CHRONIC HYPOBARIC HYPOXIA: PHYSIOLOGICAL IMPLICATIONS FOR EXERCISE PERFORMANCE

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This research programme was carried out in collaboration with the University of Oxford (UK), University of Loughborough (UK), University of New Mexico (USA), Witwatersrand Medical School (S.Africa) and the British Olympic Medical Centre (UK)

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Certificate of Research

This is to certify that, except where specific reference is made, the work described in this thesis is the result of my own work. The research was conducted at the University of Glamorgan (UK), British Olympic Medical Centre (UK), University of Oxford (UK), University of Loughborough (UK), University of New Mexico (USA) and Witwatersrand Medical School (S.Africa).

Neither this thesis, nor any part of it, has been presented, or is currently submitted, in candidature for any degree at any other University.

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ABSTRACT

Acclimatisation to a decreased inspiratory partial pressure of oxygen (P_IO_2) initiates a series of physiological adaptations that influence oxygen transport and utilisation. Whilst it is clear that adequate acclimatisation is necessary to achieve optimal physical performance at altitude, scientific evidence to support the potentiating effects following return to sea-level is at present equivocal due to the inconclusive findings of a large number of uncontrolled studies. Previous research has focused on the optimisation of the theoretically beneficial aspects of altitude acclimatisation. However, not all aspects of altitude acclimatisation are beneficial which has important implications for the health and fitness of the elite competitor.

Two separate investigations were conducted to determine the physiological implications of 4 weeks of moderate altitude training at New Mexico, USA or Krugersdorp, S.Africa (1,500 m to 2,000 m) for selected indices of submaximal, maximal and supramaximal running performance at altitude and following return to sea-level.

Resting haemoglobin concentration (Hb) did not change at altitude or following return to sea-level. An insufficient hypoxic stimulus (intensity and duration) and/or the already depressed iron stores that were observed at sea-level (serum ferritin concentration: 48 ± 35 $ng.ml^{-1}$) may have been implicated in the general lack of haematological adaptation.
In contrast, chronic hypobaric hypoxia (~P_IO_2 of 115 mmHg to 125 mmHg) was associated with adverse changes in immune function. A significant decrease in resting plasma glutamine concentration ($P < 0.001$ vs pre-altitude mean) may have been implicated in an increased frequency of infectious illnesses (upper respiratory and gastrointestinal tract infections: URTI/GTI) observed at altitude. Two male subjects who had contracted an URTI/GTI during the New Mexico sojourn were subsequently diagnosed with infectious mononucleosis shortly following return to sea-level. The evidence would suggest that these subjects were exposed to the Epstein-Barr virus during the initial stages of acclimatisation, presumably when they were most susceptible to antigenic invasion. The physical symptoms of one male subject who contracted an infectious illness during the S.Africa sojourn continued to persist even 17 months following return to sea-level.

Physiological performance during and following recovery from both maximal and supramaximal exercise was affected at altitude probably due to a more pronounced alveolar-end-capillary diffusion limitation. Supramaximal running velocity during a track session decreased by 3 to 4% at 1,500 m to 1,640 m ($P < 0.05$). Running time to exhaustion during a maximal exercise test decreased by 21% at 1,640 m ($P < 0.05$ vs pre-altitude mean). Maximal heart rate was 12 $b.min^{-1}$ lower at altitude ($P < 0.01$) and despite a 31.2 $L.min^{-1}$ ($P < 0.01$) increase in maximum minute ventilation ($\dot{V}_E$ STPD), maximal oxygen consumption ($\dot{V}O_{2max}$) expressed in both absolute and relative terms decreased by 14% ($P < 0.05$).

The lactate threshold and other cardiorespiratory determinants of submaximal and maximal running performance at sea-level were not improved by altitude training. In contrast, supramaximal running velocity decreased by 2% ($P < 0.05$) following 3 weeks return to sea-level in the altitude-trained group only.

In conclusion, the present research findings suggest that the elite athlete who trains at altitude is more susceptible to physical injury and infectious illness which may have a negative impact on physiological performance following return to sea-level. The potentially adverse effects of chronic hypoxia and the subsequent implications for the health and fitness of the elite competitor need to be considered if altitude training is to be incorporated into an elite athlete's training programme.

ACKNOWLEDGMENTS

Philosophy begins when men are perplexed. At first they puzzle about things near at hand, then gradually extend their questioning to greater matters. A man who is puzzled and amazed recognises his own ignorance. Thus, since men turned to philosophy in order to escape from a state of ignorance, their aim was evidently understanding rather than practical gain.

Metaphysics, $I._{\Pi}$. - Aristotle

It is with these words that I would like to acknowledge the contributions of Professor Bruce Davies, my Director of Studies and a personal friend. Bruce has been a constant source of energy who has instilled in me the qualities of scientific inquiry, the ability to *puzzle*, the need for *self-criticism* and ultimately the drive and desire to *understand*. These qualities have transcended not only my scientific research but all aspects of my life.

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Finally, I would like to dedicate this thesis to my grandfather, the late Graham Miles Jenkins whose memory is a constant source of inspiration:

FAREWELL TO A FRIEND

I imagined your pained expression, frightened, bewildered as you fell,
Like a Sun, in his last deep hour;
Imagined the magnificent recession of farewell,
Clouding, half gleam, half glower,
And a last splendour burn the heavens of your cheek.
And in your eyes
The cold stars lighting, still young, not weak
In different skies.

How far even then mathematics will suffice to describe, and physics to explain, the fabric of the body, no man can foresee. It may be that all the laws of energy, and all the properties of matter and all the chemistry of all the colloids are as powerless to explain the body as they are impotent to comprehend the soul. For my part, I think it is not so. Of how it is that the soul informs the body, physical science teaches me nothing; and that living matter influences and is influenced by mind is a mystery without a clue. Consciousness is not explained to my comprehension by all the nerve-paths and neurones of the physiologist; nor do I ask of physics how goodness shines in one man's face, and evil betrays itself in another. But of the construction and growth and working of the body, as of all else that is of the earth earthly, physical science is, in my humble opinion, our only teacher and guide.

D'Arcy Wentworth Thompson,
On Growth and Form, 1917.

COMMON ABBREVIATIONS

Respiratory:

ATPS - Ambient temperature pressure saturated

BTPS - Body temperature pressure saturated

STPD - Standard temperature pressure dry

F_IO_2 - Fraction of inspired oxygen (%)

PO_2 - Partial Pressure of oxygen (mmHg)

P_IO_2 - Partial pressure of inspired oxygen (mmHg)

PAO_2 - Alveolar partial pressure of oxygen (mmHg)

PaO_2 - Arterial partial pressure of oxygen (mmHg)

PCO_2 - Partial Pressure of carbon dioxide (mmHg)

$PACO_2$ - Alveolar partial pressure of carbon dioxide (mmHg)

$PaCO_2$ - Arterial partial pressure of carbon dioxide (mmHg)

$[A\text{-}a]O_2$ - Alveolar to arterial partial pressure of oxygen difference (mmHg)

CaO_2 - Arterial oxygen content ($mlO_2.dl^{-1}$).

SaO_2 - Arterial oxygen saturation (%)

P_{50} - Partial pressure for 50% saturation of haemoglobin with oxygen (mmHg)

$\dot{V}O_2$ - Oxygen uptake ($L.min^{-1}$/ $ml.kg.^{-1}$ min^{-1}/ $ml.kg.^{-0.75}min^{-1}$)

$\dot{V}O_{2rm}$ - Oxygen uptake of respiratory muscles ($L.min^{-1}$)

$\dot{V}O_{2max}$ - Maximal oxygen uptake ($L.min^{-1}$/ $ml.kg.^{-1}$ min^{-1}/ $ml.kg.^{-0.75}min^{-1}$)

$\dot{V}_E$ - Minute ventilation expressed in $L.min^{-1}$ (BTPS or STPD)

$\dot{V}_E/\dot{V}O_2$ - Ventilatory equivalent for oxygen ($L.min^{-1}$ STPD)

$\dot{V}CO_2$ - Carbon dioxide output ($L.min^{-1}$ STPD)

RER - Respiratory exchange ratio

FVC - Forced vital capacity ($L.min^{-1}$ BTPS)

FEV_1 - Forced expiratory volume in 1 second ($L.min^{-1}$ BTPS)

PEF - Peak expiratory flow rate ($L.min^{-1}$ BTPS)

$FEF_{25\text{-}75\%}$ - Mid-expiratory flow rate ($L.min^{-1}$ BTPS)

MVV - Maximum Voluntary Ventilation ($L.min^{-1}$ BTPS)

MSV - Maximum sustainable ventilation ($L.min^{-1}$)

HVR - Hypoxic ventilatory response ($L.min^{-1}\%^{-1}$)

$\dot{V}_A/\dot{Q}_C$ - Ventilation-perfusion ratio

PCTT - Pulmonary capillary transit time (s)

EIA	- Exercise induced asthma
EIB	- Exercise induced bronchospasm
URTI	- Upper respiratory tract infection

Cardiovascular:

ECG	- Electrocardiogram
HR	- Heart rate ($b.min^{-1}$)
MAHR	- Maximum attainable heart rate ($b.min^{-1}$)
ΔHR	- Delta heart rate (Standing value minus supine value, $b.min^{-1}$)
RPE	- Rating of perceived exertion (Borg, 1973)
SV	- Stroke volume (ml)
PV	- Plasma volume (ml)
$\dot{Q}$	- Cardiac output ($L.min^{-1}$)
Vc	- Capillary blood volume (ml)
SBP	- Systolic blood pressure (mmHg)
DBP	- Diastolic blood pressure (mmHg)
MABP	- Mean arterial blood pressure (mmHg)
TPR	- Total peripheral resistance ($mmHg.L.^{-1}min$)

Metabolic:

ATP	- Adenosine triphosphate ($mmol.kg.^{-1}$ dry muscle)
ADP	- Adenosine diphosphate ($mmol.kg.^{-1}$ dry muscle)
AMP	- Adenosine monophosphate ($mmol.kg.^{-1}$ dry muscle)
IMP	- Inosine monophosphate ($mmol.kg.^{-1}$ dry muscle)
Pi	- Phosphate ($mmol.kg.^{-1}$ dry muscle)
PNC	- Purine nucleotide cycle
NH_3	- Ammonia concentration ($\mu mol.L^{-1}$)
NH_4+	- Ammonium ions
$[La^-]_B$	- Whole blood lactate concentration ($mmol.L^{-1}$)
$\theta\ [La^-]_B$	- Lactate threshold ($L.min^{-1}$)
HCO^-_3	- Bicarbonate concentration ($mmol.L^{-1}$)
Hb	- Haemoglobin concentration (g/dl)
PCV	- Packed cell volume (L/L)

EPO	- Erythropoietin concentration ($mUml^{-1}$)
2,3-DPG	- Erythrocyte 2,3-Diphosphoglycerate (μmol)
HIF_{-1}	- Hypoxia-inducible factor 1
BCAA	- Branched chain amino acids
5-HT	- 5-hydroxytryptamine ($nmol.g^{-1}$)
FFA	- Free fatty acid (albumin bound) concentration ($\mu eq.L^{-1}$)
TBARS	- Thiobarbituric reactive substances ($nm.g^{-1}$ Hb)
EDRF	- Endothelium-derived relaxing factor
NO	- Nitric oxide
TC	- Total cholesterol ($mmol.L^{-1}$)
Tg	- Triglycerides ($mmol.L^{-1}$)
VLDL	- Very low density lipoprotein ($mmol.L^{-1}$)
IDL	- Intermediate density lipoprotein ($mmol.L^{-1}$)
LDL	- Low density lipoprotein ($mmol.L^{-1}$)
HDL	- High density lipoprotein ($mmol.L^{-1}$)
Lp (a)	- Lipoprotein (a) ($mg.dl^{-1}$)
Apo A	- Apolipoprotein A ($mg.dl^{-1}$)
Apo B	- Apolipoprotein B ($mg.dl^{-1}$)
EBV	- Epstein-Barr Virus
BMNC	- Blood mononuclear cells
CETP	- Cholesterol ester transfer protein

Enzyme activities ($\mu kat.g^{-1}$ dry muscle):

CS	- Citrate synthase
HAD	- 3 hydroxy-Coacyl dehydrogenase
ASAT	- Aspartate aminotransferase
SDH	- Succinate dehydrogenase
MD	- Malate dehydrogenase
PFK	- Phosphofructokinase
LD	- Lactate dehydrogenase
CK	- Creatine kinase
HK	- Hexokinase
Phosph	- Phosphorylase

AMP deaminase - Adenosine monophosphate deaminase

LPL - Lipoprotein lipase

LCAT - Lecithin:cholesterol acyltransferase

HTGL - Hepatic triglyceride lipase

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CHAPTER 1

INTRODUCTION

1.1 EVOLUTION AND THE IMPORTANCE OF OXYGEN (O_2)

It has been suggested that approximately 4,600 million years ago, Earth's primordial atmosphere was a reducing one which did not contain free oxygen (Dickerson, 1978). Modern day theorists believe that the major constituents of the original atmosphere were mainly hydrogen containing gases such as methane (CH_4), ammonia (NH_3) and water vapour (H_20), with a small amount of nitrogen [(N_2) Asimov, 1984]. Ultraviolet radiation that encountered water molecules in the upper atmosphere would, through a process known as photodissociation yield hydrogen and oxygen. The hydrogen would escape leaving oxygen behind, which would react with methane to form carbon dioxide (CO_2) and H_20. Thus, over time, a new atmosphere composed of mainly CO_2 and N_2 would be formed which is similar to that found today on Venus and Mars.

This process of chemical evolution preceded the evolution of life itself. Blue green algae evolved about 2500 million years ago and thus the process of photosynthesis. Slowly, the oxygen content of the atmosphere began to rise until by about 1000 million years ago the atmosphere contained 1 to 2% oxygen. This proved a sufficient energy supply for animal cells and evolutionary change shifted towards increased complication during the Phanerozoic eon with the development of multicellular organisms about 700 million years ago (Valentine, 1978).

The colonisation of dry land may be considered as the single greatest victory achieved over the inanimate environment. Fish that belonged to the subclass Crossopterygii (fringed fins) invaded land as a result of competition for oxygen in stretches of fresh water (Asimov, 1984). Natural selection favoured those fish that could make use of the abundant supply of O_2 in the atmosphere, and they developed pouches in their alimentary canals to store swallowed air (Asimov, 1984). It is believed that the human lung has evolved from these pouch like structures. Through the complicated process of evolutionary change came the primates which can be traced some 60 to 70 million years ago. *Australopithecus afarensis*, commonly known as "Lucy" marked the beginning of the history of the bipedal hominid about 4 million years ago from which modern man (*Homo sapiens sapiens*) descended and has inhabited Earth for the last 35,000 years (Astrand, 1994).

Evolutionary change has been driven by the presence of oxygen and it is only during the last one tenth of the Earth's existence that an oxygenated atmosphere has become a characteristic feature. Contemporary estimates have suggested that the green plants of the earth combine a total of 150 billion tons of carbon (from CO_2) with 25 billion tons of hydrogen (from water) to liberate 400 billion tons of oxygen each year (Asimov, 1984). Although O_2 has been present in the atmosphere since the beginning of the Earth's history, its discovery had to wait until 1774 following experiments conducted by an English chemist, Joseph Priestley.

1.2 THE DISCOVERY OF OXYGEN

The Greeks were the first to consider air as an element (Asimov, 1984). In the first century AD, the Greek engineer Hero wrote in a book entitled Pneumatics; "Vessels which seem to most men empty are not empty, as they suppose, but full of air..... composed of tiny particles minute and light and for the most part invisible..... If then, we pour water into an apparently empty vessel, air will leave the vessel proportional in quantity to the water which enters it.....Hence it must be assumed that air is matter."

Van Helmont in 1620 was the first investigator to employ the term "gas;" a derivative of "chaos" which is a Paracelsian term referring to the original substance out of which the Universe was made (Asimov, 1984). He discovered the first gases which he termed "gas sylvestre" (CO_2) which he demonstrated would extinguish a candle flame and "gas pingue" (a mixture of hydrocarbons) which would result in combustion. In 1700, George Ernst Stahl proposed the "phlogiston theory" which provided an explanation for the process of combustion (Asimov, 1984). He suggested that substances were capable of burning because they contained phlogiston (derived from a Greek word meaning to "set on fire"). In 1774, Joseph Priestley advanced this theory and it is he who is credited with the discovery of "dephlogisticated air" or oxygen. Later that year however, Laurent Antoine Lavoisier who was a French chemist, challenged the phlogiston theory. He demonstrated in a series of quantitative experiments that phlogiston did not exist and all combustion occurred as a result of the combination of air with the substance that burned in it. Following subsequent experimentation he concluded that air was composed of two independent "airs". He called these oxygene from the Greek word meaning "producer of

sourness" and the other he termed "azote" from the Greek word meaning "no animal life". The latter gas became known as nitrogen since it formed a constituent part of the common mineral niter (Asimov, 1984).

1.3 OXYGEN AND ENERGY METABOLISM

In the absence of O_2, the human organism is capable of generating energy in the form of adenosine triphosphate (ATP) anaerobically. The anaerobic pathway is the oldest metabolic pathway which has existed for at least 3500 million years, but in comparison to the aerobic system, it is comparatively inefficient (Astrand, 1994). In 1931, the German biochemist Otto Heinrich Warburg was awarded the Nobel prize in Physiology and Medicine for his research which emphasised the importance of oxygen for the survival of the human organism (Asimov, 1984). He devised the first instrument for the measurement of oxygen uptake which became known as the Warburg Manometer. This early research demonstrated that the metabolic process involved a continuous flow of oxygen to the respiring tissues. This movement is determined by the general law of transport which states that flow is proportional to a concentration or pressure difference and to a proportionality constant having the dimension of a conductance (Lenfant et al 1971). In the human organism, gas transport is achieved by four linked transport mechanisms that constitute the oxygen cascade system, which ultimately transports oxygen from the atmosphere to the mitochondria. This law can be expressed as:

$$\dot{V}\mathbf{O_2} = \dot{V}_A(\Delta \mathbf{P_1})\mathbf{k_1} + \mathbf{D_L}(\Delta \mathbf{P_2})\mathbf{k_2} + \dot{Q}(\Delta \mathbf{P_3})\mathbf{k_3} + \mathbf{D_t}(\Delta \mathbf{P_4})\mathbf{k_4}$$

In which;

$\dot{V}\mathbf{O_2}$ = oxygen uptake

$\dot{V}_A$, $\mathbf{D_L}$, $\dot{Q}$, $\mathbf{D_t}$ = alveolar ventilation, lung diffusion, blood flow and tissue diffusion conductances

$\Delta \mathbf{P}_{1,2,3,4}$ = pressure gradients corresponding to each link

$\mathbf{k}_{1,2,3,4}$ = factors determined by the appropriate dimensions

However, the metabolic process is impaired during acute exposure to a significantly reduced partial pressure of oxygen (PO_2) caused by environmental influences or disease.

Acclimatisation to a chronically reduced PO_2 invokes a series of physiological adaptations that strive to maintain adequate tissue oxygenation (Connett et al 1990). These adaptations are similar to those induced by endurance training and include a compensatory polycythaemia due to increases in packed cell volume and haemoglobin (Berglund, 1992), a shift in oxygen binding to haemoglobin to optimise arterial O_2-loading and peripheral O_2-unloading (Mairbaurl, 1993), increases in mean muscle fibre cross sectional area and mitochondrial volume (Desplanches et al 1993), increases in tissue myoglobin and aerobic enzyme concentrations (Terrados et al 1990), intramuscular bicarbonate stores (Mizuno et al 1990, Favier et al 1995) and a shift in substrate mobilisation to predominantly free fatty acids which results in a glycogen sparing effect (Young et al 1987).

Thus, it has been suggested that altitude training may compound the normal physiological adaptations induced by exercise training and accelerate performance improvements following return to sea-level. However, there is emerging evidence which suggests that the additive stress of hypoxia *per se* may provoke adverse changes in immune function and further potentiate free radical mediated oxidative damage (Meehan et al 1988; Simon-Schnass, 1994). If the implications of these less favourable responses are confirmed by scientific rigour, they would certainly present a threat to both the fitness and health of the elite competitor who trains at altitude.

Therefore, the aims of this thesis were to examine the implications of chronic exposure to hypobaric hypoxia (4 weeks of intensive training at 1,500 to 2,000 m) for physiological indices of submaximal, maximal and supramaximal exercise performance at altitude and following return to sea-level. The subjects employed in these investigations were elite middle to long distance runners, who were born and bred at, or close to sea-level in the UK.

This thesis contains six chapters which are briefly outlined below:

Chapter 2:

The review of the literature will address the major atmospheric changes that occur with increasing height above sea-level with the focus on changes in barometric pressure and subsequent implications for the inspired partial pressure of oxygen (P_IO_2). A physiological rationale for altitude training as a means of improving sea-level exercise performance will be

presented, with particular reference to blood re-infusion studies and comparative investigations between the native lowlander and the native highlander. The metabolic and musculocardio-respiratory responses invoked by acute and chronic exposure to hypoxia per se at rest and during exercise will be examined. Finally, the effects of hypoxic training on sea-level endurance performance will be summarised with a discussion of the potential factors that may modulate this response.

In light of present research findings, this chapter will conclude with the formulation of a series of experimental aims and null hypotheses.

Chapter 3:

Theoretical and Methodological Background will describe the medical and physiological testing procedures and experimental apparatus employed during the research. Reference will be made to a number of "quality control" experiments which were conducted to maximise experimental validity. These experiments are appended (Appendix E-G and I-K).

Chapter 4:

Altitude study 1: This chapter will outline a study which examined the effects of chronic hypobaric hypoxia (~1,500-2,000 m) on physiological indices of submaximal and supramaximal running performance at altitude and following 21 days return to sea-level. The altitude training camp was based in Albuquerque, New Mexico, U.S.A.

Chapter 5:

Altitude study 2: As a follow-up to Study 1, this chapter will describe a study which was designed to quantify the physiological implications of chronic hypobaric hypoxia (~1,640 m) on maximal and supramaximal indices of exercise performance at altitude and following 10 and 21 days return to sea-level. The altitude training camp was based in Krugersdorp, S.Africa.

Chapter 6:

The final chapter, entitled General Discussion will briefly outline the limitations of the experimental research described in this thesis. The realisation of aims will be considered prior to an examination of the null hypotheses that were formulated in conclusion to present

research findings that were discussed in Chapter 2. A general discussion will integrate and interpret the research findings of the present study and consider the physiological implications of altitude training for the health and fitness of the elite competitor. Finally, a series of altitude-training guidelines will be proposed as a means of *optimising* the physiological acclimatisation process.

CHAPTER 2

REVIEW OF THE LITERATURE

2.1 SUMMARY

The review of literature compromises eleven sections.

The first section (Section 2.2) will examine the major physiological components of endurance performance.

Section 2.3 will discuss the atmospheric changes that occur with an increase in altitude and subsequent implications for exercise performance.

Section 2.4 presents a physiological rationale for the use of hypoxic training as a legal means of enhancing exercise performance. Particular reference will be made to the ergogenic benefits of secondary polycythaemia. Investigations that have artificially induced erythrocythaemia via autologous blood reinfusion or following administration of recombinant human erythropoietin and subsequent implications for exercise performance will be reviewed.

Comparative investigations that have studied the physiological attributes of populations born and raised at altitude (native highlanders) will also be discussed. The hypothesis that hypoxia *per se* is responsible for a number of central and peripheral adaptations that facilitate oxygen transport and utilisation will be critically examined.

Section 2.5 will discuss the physiological implications of acclimatisation (chronic versus acute exposure) to environmental hypoxia for exercise performance in the native lowlander. The responses following return to sea-level will also be examined.

Section 2.6 will examine the effects of acute and chronic hypoxia on aerobic and anaerobic performance.

Altitude training as a means of enhancing physical exercise following return to sea-level will be critically reviewed in Section 2.7.

Section 2.8 will critically examine the factors that affect exercise performance following return to sea-level. This section will discuss the major dietary and methodological technicalities that affect the physiological responses invoked during altitude acclimatisation and how these can be optimised.

Section 2.9 will address the potentially less favourable physiological responses to hypoxic training and the subsequent implications for the health and fitness of the individual who trains at altitude.

Section 2.10 will summarise the review of literature and briefly outline future research directions.

Finally, Section 2.11 will outline the experimental aims of the present research and will conclude with the presentation of the null hypotheses (H_O).

2.2 PHYSIOLOGICAL COMPONENTS OF DISTANCE RUNNING

The physiology of distance running involves the complex interaction between respiratory, cardiovascular and cellular energy systems. The gas transport mechanisms that link internal (cellular) and external (pulmonary) respiration are illustrated in Figure 2.2. As outlined in the previous chapter, oxygen is a fundamental substrate required to drive this mechanism and ultimately generate ATP. Thus, values for maximal oxygen uptake ($\dot{V}O_{2max}$) are characteristically high in elite endurance athletes from a variety of sporting specialities. This is illustrated in Figure 2.1 which contains data obtained from International standard athletes tested at the British Olympic Medical Centre (unpublished data).

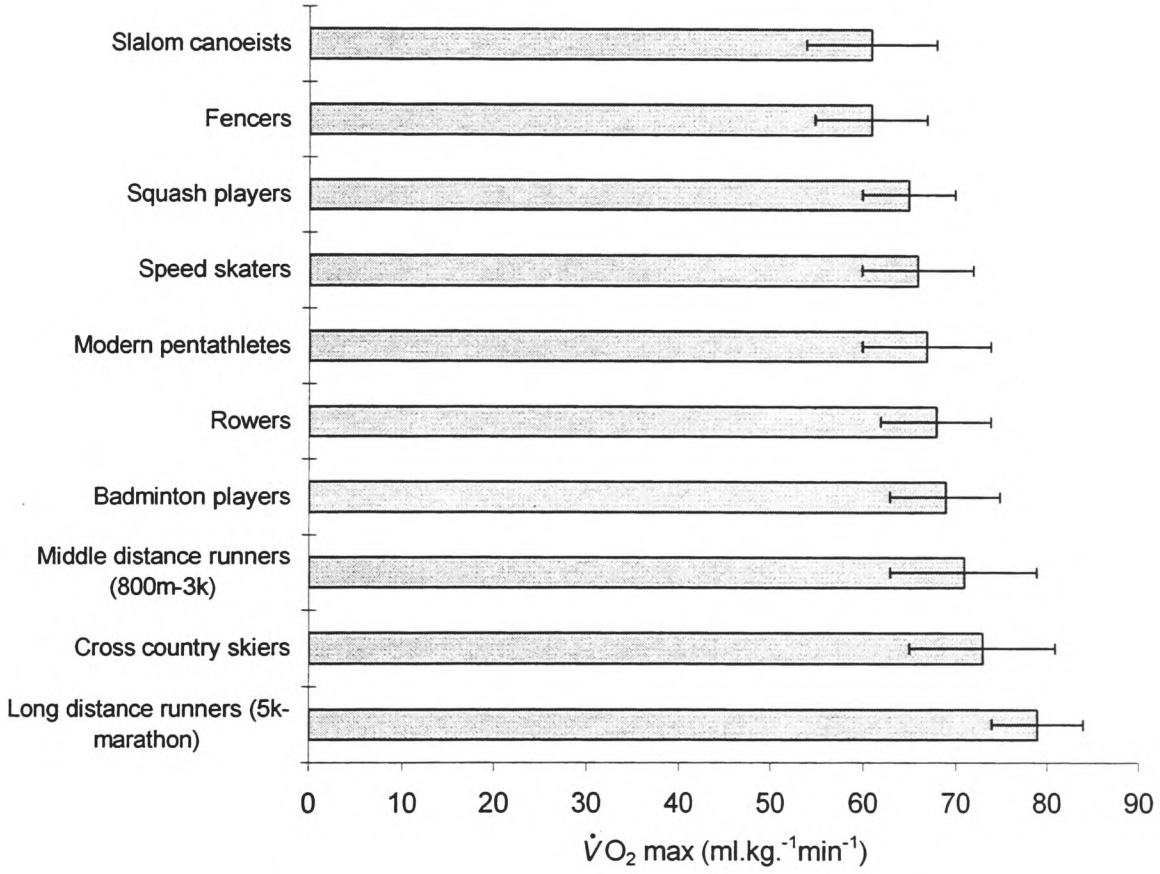


Figure 2.1 Maximal Oxygen Uptake ($\dot{V}O_{2max}$) of Elite Male Athletes
Values are Mean ± SD based on 150 subjects

Raven and Hagan (1994) have suggested that the regulation of internal and external respiration is controlled by the detection of "error" signals related to skeletal muscle oxygen demand. A disturbance of homeostasis such as a decrease in ambient PO_2 stimulates a myriad of compensatory mechanisms which serve to optimise tissue oxygenation (Figure 2.2). Whether or not such adaptations improve endurance

performance is still the subject of much debate and will be critically examined in this review.

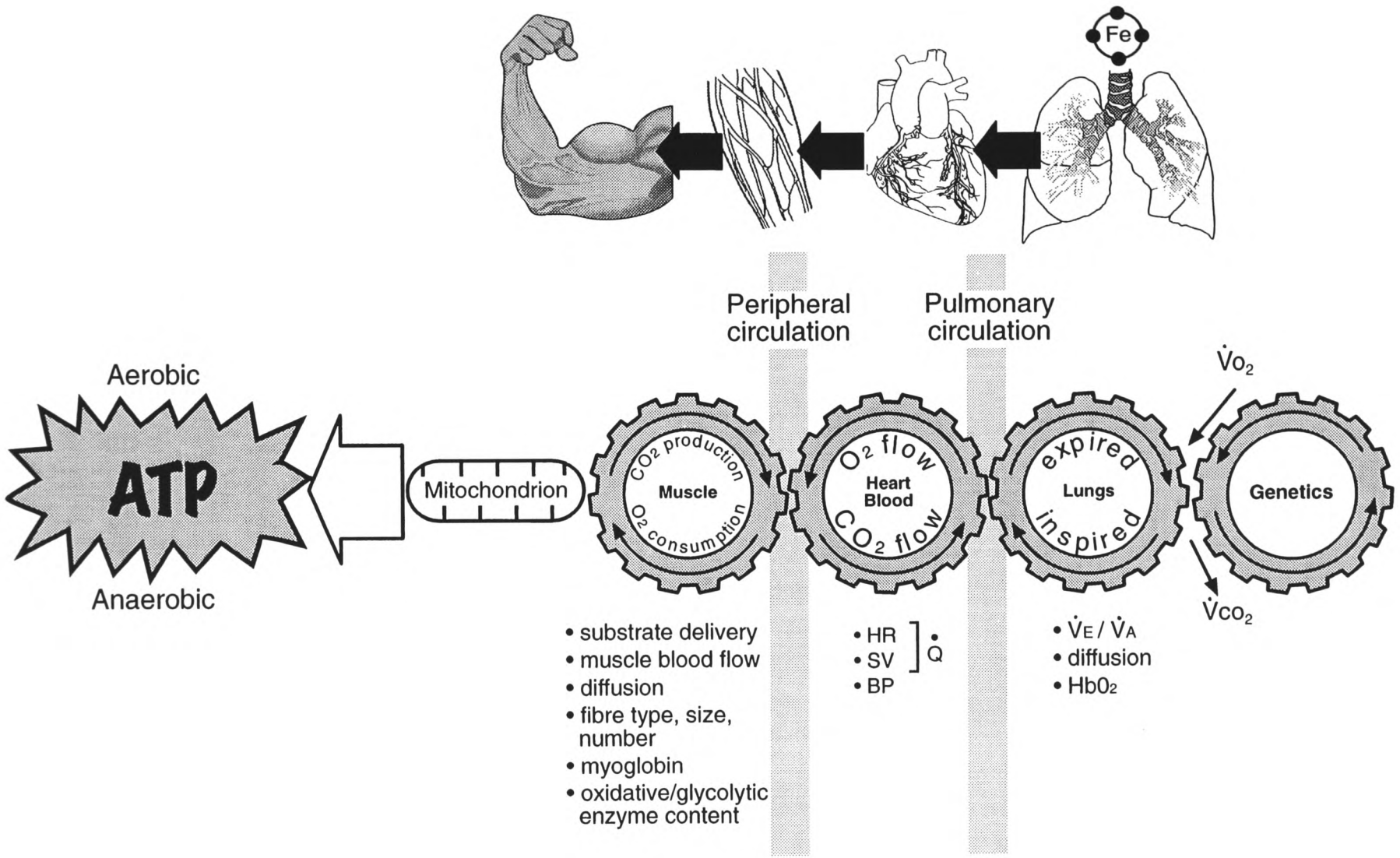


Figure 2.2 Oxygen Transport and Utilisation (modified from Wasserman, 1987)

2.3 PHYSICAL ENVIRONMENT AT ALTITUDE

Aristotle was arguably the first scientist to stimulate research into the atmosphere (Asimov, 1984). He suggested that the world was composed of four shells that constituted the four elements of matter; earth, water, air and fire. The universe beyond these shells, he proposed, was composed of an unearthly perfect fifth element that he called "ether". However, he could not accept the existence of emptiness or a vacuum, which was later discovered in 1638 by Galileo. Galileo's research stimulated subsequent investigations by his two students, Evangelista Torricelli and Vincenzo Viviani whose findings marked the beginning of our understanding of the environment at altitude and the subsequent physiological implications for exercise performance (Ward *et al*, eds, 1995).

2.3.1 Barometric Pressure

"We live at the bottom of an ocean of the element air, which by unquestioned experiments is known to have weight.....which on the tops of high mountains begins to be distinctly rare" **Torricelli (1644)**

Toriccelli's most revolutionary discovery was that the force of a vacuum was due to the weight of the atmosphere above him (hence the term *Torricellian vacuum*). He invented the first mercury barometer in 1644, which Pascal and Perier subsequently used to demonstrate a decrease in barometric pressure on the top of Puy de Dome (1463 m) in central France in comparison to measurements conducted at sea-level (Buskirk, 1996). However, if these early investigators had repeated their measurements at a variety of different altitudes, they would have discovered that the decrease in barometric pressure with increasing altitude was not linear. In fact, the relationship between barometric pressure and altitude is complicated by other factors (Ward *et al*, eds, 1995). Firstly, air is compressible and thus the decrease in barometric pressure is more pronounced nearer the earth's surface. Secondly, a decrease in temperature occurs with increasing altitude. The relationship between volume, temperature and the pressure of gases was demonstrated by Boyle and Charles whose findings culminated in the ideal gas law which demonstrates that at a constant temperature, the pressure of a given mass of gas is inversely proportional to its volume (Boyle's Law) and at constant pressure, the volume of a gas is directly proportional to its absolute temperature (Charles' Law); expressed as:

$$\mathbf{PV = nRT}$$

where:

n = number of gram molecules of the gas

R = "gas constant" equivalent to 62.4 when **P** (pressure) is expressed in mmHg, **V** (volume) in litres and **T** (temperature) in degrees Kelvin

Based on these physical constructs, Zuntz et al. (1906) developed an equation which has provided the most accurate mathematical description to date of the relationship between barometric pressure and altitude. However, its accuracy is limited in that the mean air column temperature is assumed to be +15°C. This is a clear overestimation of the actual temperature at high altitude which for example is known to be -9°C on the summit of Mt Everest in October (West et al 1983b).

$$\log b = \log B - \frac{h}{72(256.4 + t)}$$

where:

b = barometric pressure at higher altitude (mmHg)

B = barometric pressure at the lower altitude (mmHg)

h = altitude difference in metres

t = mean temperature (°C) of the air column of height h

Figure 2.3 illustrates the relationship between barometric pressure and altitude according to calculations based on the Zuntz et al. (1906) formula. This relationship is compared with calculations based on the standard atmosphere which were developed by the International Civil Aviation Organization (ICAO, 1964) for the calibration of aircraft altimeters (Ernsting and King, eds, 1995). The standard atmosphere assumes a sea-level barometric pressure of 760 mmHg, a temperature of +15°C, and a linear decrease in temperature with altitude (lapse rate) of 6.5°C per kilometre (km) up to an altitude of 11km. However, Haldane and Priestly (1935) demonstrated that the standard atmosphere predicted barometric pressures considerably lower than those determined by Zuntz et al. (1906). Following direct measurements of barometric pressure in the Himalayas during the pre-monsoon period by Pugh (1957) and later by West et al. (1983b), it was concluded that the decrease in barometric pressure with altitude followed the relationship predicted by Zuntz

et al. (1906) better than the standard atmosphere. Although the differences in these calculations are insignificant up to altitudes of 5500 m the physiological implications for the underestimation of barometric pressure on the summit of Mt Everest (8848 m) according to the ICAO model are profound and would have made the task of conquering the summit of Mt Everest in the Winter without supplementary oxygen impossible (West, 1983).

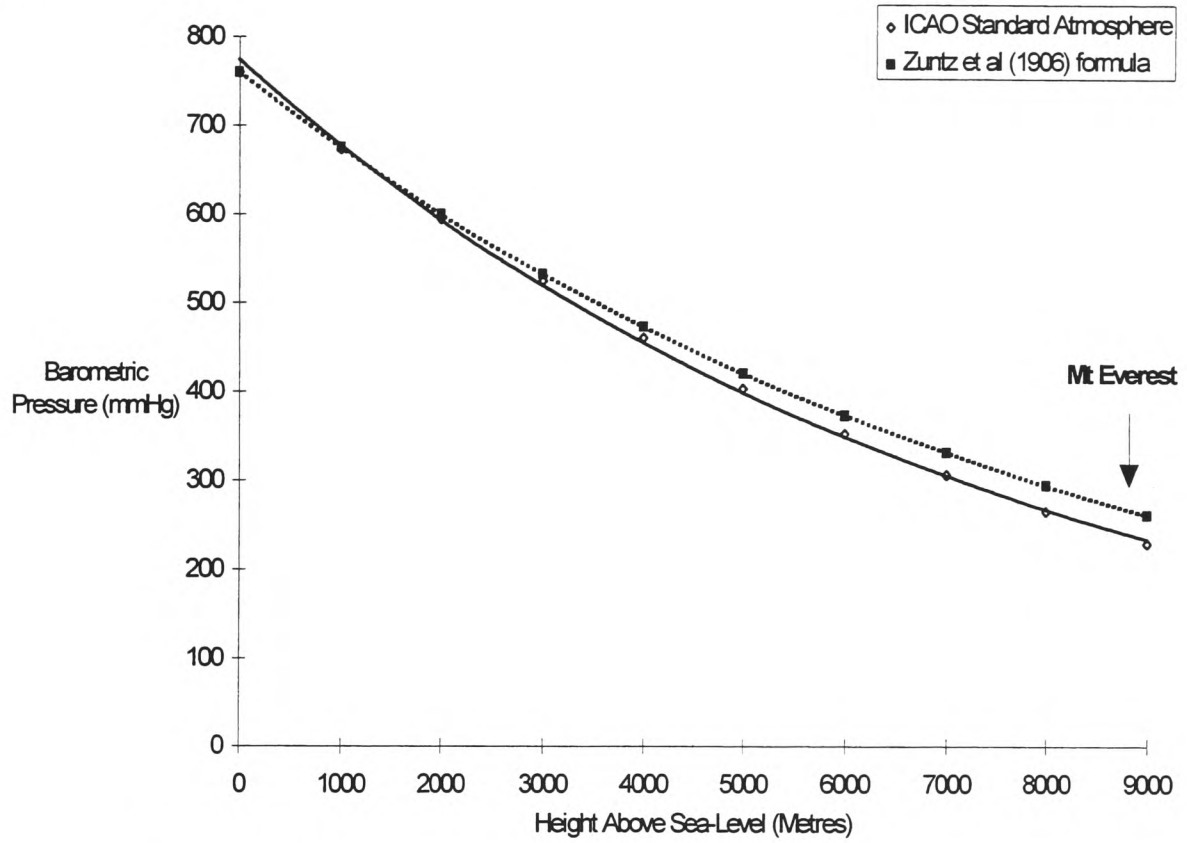


Figure 2.3 Barometric Pressure at Altitude

2.3.2 Partial Pressure of Inspired Oxygen (P_IO_2) at Altitude

By the mid nineteenth century, the French chemist Henri Victor Regnault discovered that the composition of the atmosphere was the same all over the world and at any given altitude (Asimov, 1984). He demonstrated that air contained 20.94% oxygen, 79% nitrogen, 0.03% carbon dioxide with small traces of argon and other trace gases. The contribution that each gas makes to the total barometric pressure is termed the partial pressure. According to Dalton's Law, in a gas mixture, the pressure exerted by each individual gas is independent of the pressures of the other gases in the mixture. The partial pressure of an individual gas is equal to its fractional concentration times the total pressure of all the gases in the mixture (Levitzky, 1995).

Expressed mathematically, the law states:

$$P_{gas} = \% \text{ total gas} \times P_{total}$$

Thus the barometric pressure directly determines the ambient partial pressure of oxygen. When atmospheric air is inspired, it becomes saturated with water vapour pressure which is equivalent to 47mmHg at 37°C. Thus, calculation of the P_IO_2 can be expressed as:

$$P_IO_2 = 0.2094(P_b - 47)$$

Figure 2.4 illustrates the decreases in PO_2 for dry atmospheric air and moist inspired air with increasing altitude. These data were determined according to changes in barometric pressure calculated using the Zuntz et al. (1906) formula, assuming a mean air column temperature of +15°C and a fractional concentration of oxygen equivalent to 20.94%.

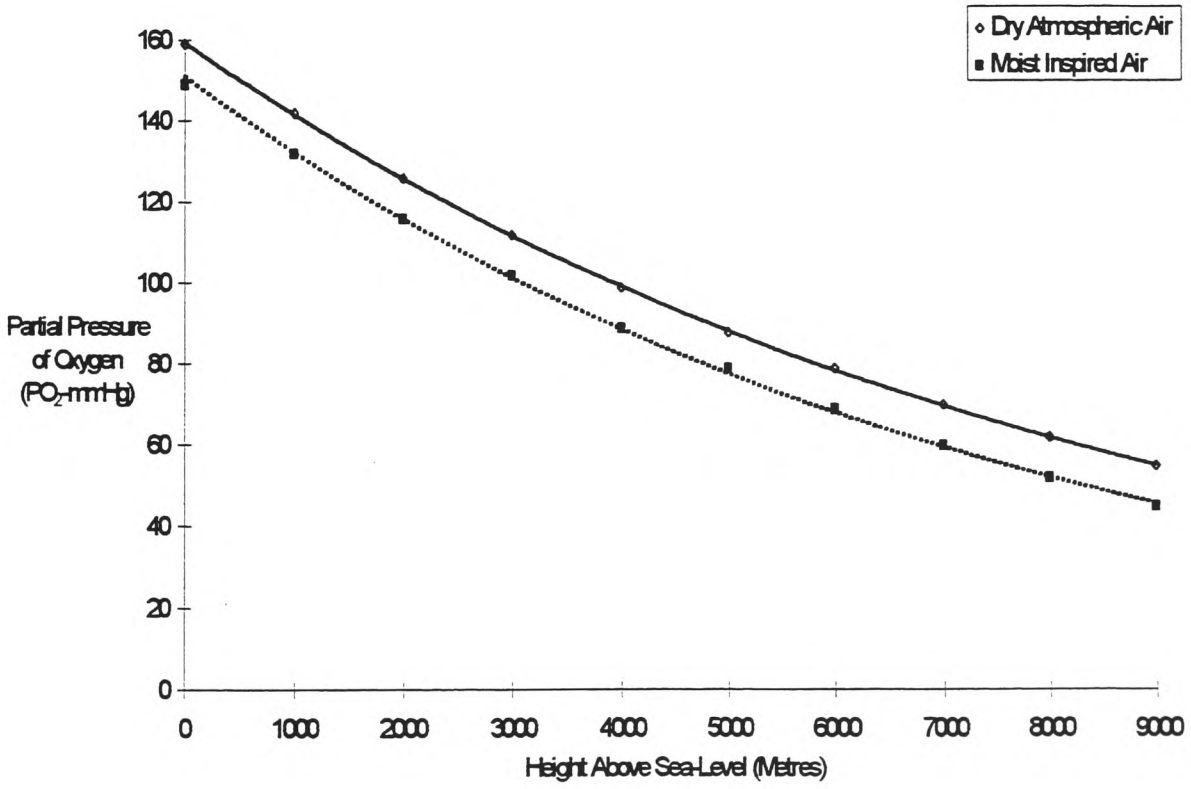


Figure 2.4 Ambient and Inspired Partial Pressure of Oxygen (PO_2) at Altitude

When one considers the deleterious effects of altitude on physical exercise, other factors in addition to a reduced P_IO_2 may also modulate exercise performance. However, the

physiological significance of these factors has been largely ignored in the majority of investigations that have studied exercise at altitude.

2.3.3 Temperature

In addition to hypoxia, the subject at altitude also encounters the additional stress of a cold climate. In comparison to sea-level conditions, ambient temperature decreases at a rate equivalent to 1.98°C per 350 m increase in altitude (Ernsting et al, eds, 1995). In addition to this, wind velocities tend to be greater at altitude, which subsequently increases the wind chill factor (Mills, 1973 and Pate, 1988).

The decrease in ambient temperature at altitude should be considered as an additional stressor that challenges musculo-cardiorespiratory and metabolic control at rest and during exercise (Giesbrecht, 1995). Bergh (1980) has clearly demonstrated adverse changes in physiological indices of submaximal and maximal exercise performance if the environmental conditions cause a decrease in body core temperature. Acute and chronic exposure to cold causes peripheral vasoconstriction which results in a diuresis activated initially by a release of atrial natriuretic peptide (Atrial natriuretic peptide, 1986). Respiratory and evaporative fluid loss also increase at altitude, due to the low absolute humidity and increase in hypoxia-mediated pulmonary ventilation (Kayser, 1994).

It has also been suggested that cold and hypoxia depress the sensation of thirst to a greater extent than that experienced at sea-level, as demonstrated by Blume et al (1984) who identified that native highlanders who were born and raised at altitude were in a chronic state of dehydration despite the availability of adequate fluids. In addition to this, direct ultraviolet radiation (290-320nm) increases at a rate of 35% per 1,000 m (Shephard, 1992). Thus, chronic altitude exposure to the sun's harmful rays increases the susceptibility to sunburn and free radical mediated oxidative stress (Simon-Schnass, 1994). Even a moderate sunburn injury has been shown to impair thermoregulatory function and thus exacerbate the dehydration process (personal communication, Dr M.Sawka, US Army Research Institute of Environmental Medicine, Natick, USA). The physiological implications of hypohydration for high intensity and endurance exercise performance has been rigorously investigated (Maughan, 1994). Saltin et al (1988) demonstrated that fluid

loss equivalent to as low as 2% body mass (1.4 L for a 70 kg man) impaired endurance performance, and losses in excess of 5% decreased work capacity by 30%.

2.3.4 Gravity and Wind Resistance

At moderate altitudes of 2000 to 4,000 m, changes in the acceleration due to gravity are insignificant and do not influence exercise performance. However, wind resistance decreases at altitude due to an exponential reduction in atmospheric density (Shephard, 1992), expressed as:

$$\textbf{Wind resistance} = {}^{1}/_{2}\,(Apv^{2})$$

where:

A - projected area of the subject

p - density of the atmosphere

v - relative velocity of air movement

The reduction in wind resistance was strongly implicated in the world record performances in several sprinting and throwing events witnessed at the 1968 Mexico Olympic Games held at 2,248 m. Peronnet et al. (1991) has produced a theoretical model based on the changes in wind resistance at altitude to predict world record times for a variety of sprinting and distance running events.

2.3.5 Summary

Thus it is clear from the previous discussion that the environment at altitude imposes other stresses which confound that of hypoxia. This may contribute to the current controversey that surrounds the efficacy of altitude training as a means of enhancing sea-level endurance performance. Changes in temperature, humidity, wind velocity and air density all have important implications for exercise performance which have been largely ignored by *mountaineering studies* that have focused primarily on the physiological responses to a decreased ambient PO_2. Studies conducted in hypobaric chambers such as Operation Everest II have significantly improved our understanding of the physiological adaptations invoked during altitude acclimatisation (Houston et al 1987) due to the improved control

over the environment. Throughout this review, reference will be made to both field and chamber studies and where applicable, the limitations of these studies will be discussed.

2.4 PHYSIOLOGICAL RATIONALE FOR ALTITUDE TRAINING

2.4.1 Introduction

Quantitative research into hypoxia began with the advent of ballooning in 1783 and experiments conducted in a hypobaric chamber by Paul Bert (Buskirk, 1996). Paul Bert's classical publication *"La Pression Barometrique"* in 1878 demonstrated that acclimatisation to a chronically reduced inspiratory partial pressure of oxygen (P_IO_2) invoked a series of central and peripheral adaptations that served to maintain adequate tissue oxygenation in healthy skeletal muscle. These physiological adaptations have been subsequently implicated in the improvement in exercise performance during altitude acclimatisation.

However, it wasn't until the 1950's that scientists suggested that the additive stimulus of environmental hypoxia could potentially compound the normal physiological adaptations to endurance training and accelerate performance improvements following return to sea-level (Hollmann, 1994). Subsequent experiments conducted under conditions of normobaric hyperoxia have clearly demonstrated that healthy skeletal muscle *has the potential* to utilise more oxygen than that provided during exercise under conditions of normoxia. The implications of normobaric hyperoxia for athletic performance are profound. Welch (1987) summarised the linear relationship between increases in P_IO_2 and endurance time to exhaustion, so that performance improvements of up to 40% have been recorded at a P_IO_2 of 800 mmHg. However, there are alternative components, in addition to increasing the fractional PO_2 of inspired gases that can also facilitate oxygen transport and utilisation (Figure 2.1).

The continued popularity of altitude training has been influenced by two factors. First hypoxia *per se* increases blood haemoglobin concentration, which has been demonstrated to improve endurance performance. Secondly, several of the best endurance runners in the World have originated from East African countries that are based at altitudes of 1500 to 2000 m. Is it possible that either living and/or training at altitude may contribute to their running success?

2.4.2 Autologous Blood Reinfusion and Endurance Performance.

One of the most documented physiological adaptations to a reduced P_IO_2 is the increased release of erythropoietin (EPO) which causes a transient increase in red blood cell mass (Schmidt et al 1993). The implications of secondary polycythaemia to both submaximal and maximal indices of endurance performance at sea-level have been demonstrated by studies that have artificially induced erythrocythaemia following either autologous blood reinfusion (Gledhill, 1985 and Sawka et al 1996) or by subcutaneous injections of recombinant human erythropoietin (Ekblom et al 1991 and Metra et al 1991). The major research findings are summarised in Table 2.1.

Table 2.1 Effects of Autologous Blood Reinfusion on Maximal Oxygen Uptake ($\dot{V}O_{2max}$)

Author/ Year	Volume of Blood reinfused (mls)	% Change in Hb following reinfusion	% Change in $\dot{V}O_{2max}$ following reinfusion
Buick '80	900	+8‡	+5‡
Spriet '80	1200	NR	+7†
Williams '81	920	+7‡	NR
Goforth '82	760	+4†	+11‡
Robertson '82	750	+28†	+13†
Thompson '82	1000	+12†	+11†
Robertson '84	475	+16†	+10†
Celsing '87	2250	+11	+7‡
Sawka '87	600	+10†	+11†
Robertson '88	475	+16†	+10‡
Ekblom '91	1350	+9†	+8†

† - Significantly different prior to reinfusion ($P < 0.05$)
‡- Significantly different prior to reinfusion ($P < 0.01$)
NR - Not reported

The ergogenic benefits of artificially induced polycythaemia are generally attributed to an increased arterial oxygen concentration (CaO_2), because at any given oxygen saturation, 1.34 to 1.39 ml of oxygen are bound per gram of haemoglobin, (Schmidt, 1980). This has the effect of displacing the oxygen dissociation curve to the right, thus reducing the oxygen

affinity of haemoglobin which facilitates the unloading of oxygen to the peripheral tissues. Whilst it has been demonstrated that 1g Hb/dl blood increases $\dot{V}O_{2max}$ by 190 ml (Celsing et al 1987) the relationship between Hb concentration and $\dot{V}O_{2max}$ is poor (r = 0.52, NS). This is illustrated in Figure 2.5.

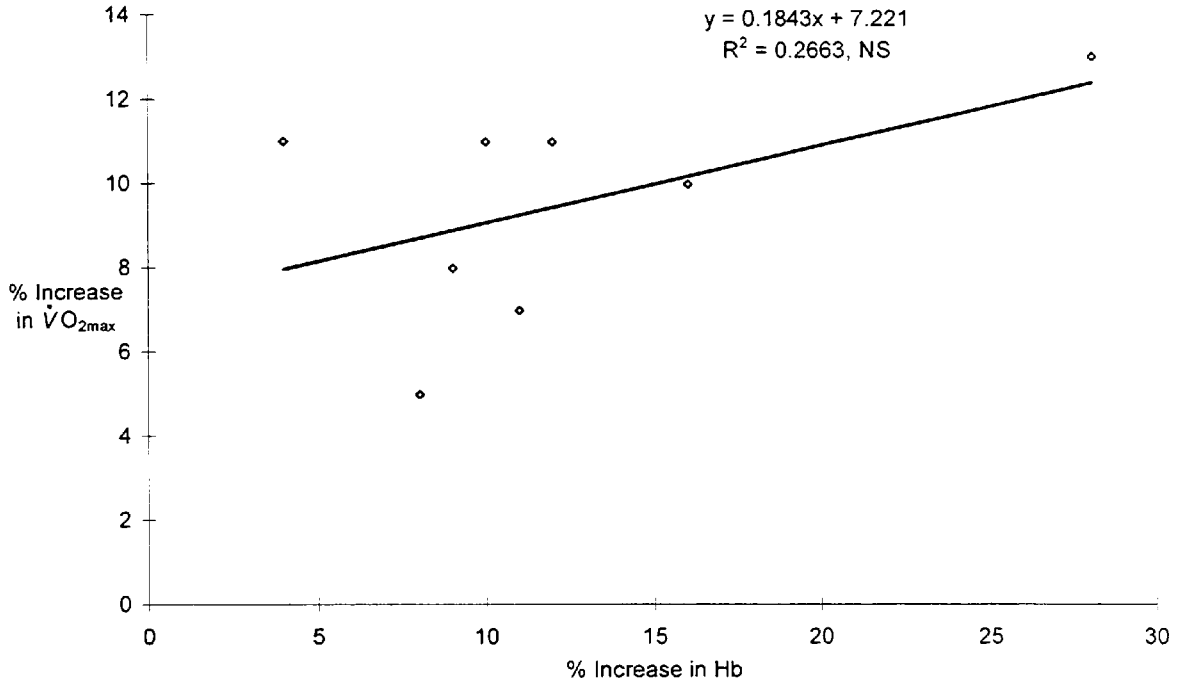


Figure 2.5 Effects of Haemoglobin (Hb) Increase Following Autologous Blood Reinfusion and Implications for $\dot{V}O_{2max}$

Values are Means
Data based on: Buick et al. (1980), Goforth et al. (1982), Robertson et al. (1982), Thompson et al. (1982), Robertson et al. (1984), Celsing et al. (1987), Sawka et al. (1987), Robertson et al. (1988), Ekblom et al. (1991).

Sawka et al. (1987) have also emphasised the variability of an individual's ergogenic response to eythrocyte infusion which they attributed to genetic factors and the subject's initial level of fitness. They later demonstrated that highly trained distance runners, ($\dot{V}O_{2max}$ ~ 70 - 90 ml.kg.$^{-1}$min.$^{-1}$) experienced the smallest improvements in $\dot{V}O_{2max}$ following blood reinfusion in comparison to moderately trained athletes (Sawka et al 1989). Several physiological mechanisms have been proposed to explain this finding, but the increases in blood viscosity would appear to be the most important factor (Mairbaurl, 1994 and Williams, 1995). Polycythaemia may limit the oxygen transport capacity of blood by reducing blood flow due to an increased vascular resistance (Lenfant et al 1971 and Schmidt, 1980) in accordance with Poiseuille's law. It is also believed that hyperviscosity may increase the susceptibility of thromb-embolic events such as stroke and

myocardial infarction (Ward et al, *eds*, 1995). The literature would suggest that the microcirculation is compromised when haematocrit and haemoglobin concentrations are raised to 55% and 18g.dl^{-1} respectively (Mairbaurl, 1994). The potential medical hazards involved has led to the practice of inducing polycythaemia (i.e. blood doping) being banned by the International Olympic Committee, (Dugal, 1976 and Sawka et al 1996).

In addition to the increases in systemic O_2 transport to skeletal muscle, it has also been suggested that an increased volume of red blood cells would facilitate blood buffering capacity (Gledhill, 1986). Lower heart rates, venous and arterial blood lactate concentrations, and higher venous and arterial pH values for a standardised sub-maximal power output have been demonstrated as a consequence of an increased red blood cell mass, (Buick et al 1980; Celsing et al 1987 and Ekblom et al 1991). Based on the rather limited number of field studies that have been conducted, it would appear that the improvement in race times following reinfusion of approximately 900 ml of autologous red blood cells, increases as a function of the distance covered, such that 1,500 to 10,000 metres race times improved on average from 7 to 68 seconds respectively (Goforth, 1982; Berglund et al 1987 and Brien et al 1987, 1989).

Erythrocyte reinfusion has also been employed to attenuate the negative physiological effects invoked during chronic exposure to heat, cold and environmental hypoxia (Sawka et al 1996). Sawka and Young (1989) suggested that the ergogenic effects of erythrocyte reinfusion would be more pronounced at altitude than at sea-level, due to the greater decrement in hypoxia-mediated $\dot{V}O_{2max}$. However, whilst artificially induced polycythaemia ameliorates the $\dot{V}O_{2max}$ decrement at moderate altitudes of between 2,225 (Robertson et al 1988) to 3,566 metres (Robertson, 1982), a recent investigation did not demonstrate performance improvements at an altitude of 4300 metres (Young et al 1996). It was clear from the latter study that erythrocyte reinfusion did not facilitate oxygen delivery to active skeletal muscle tissue despite an elevated arterial oxygen content (CaO_2). The decrement in arterial PO_2 (PaO_2) during maximal exercise was similar in the erythrocyte/saline infused groups and it was concluded that a pulmonary diffusion limitation at the higher altitudes precluded the benefits of an enhanced O_2-carrying capacity of the blood. It is also conceivable that hypoxia mediated peripheral vasoconstriction in response to an increased CaO_2 (Wolfel et al 1991) and a decreased plasma volume (Young, 1988) would obviate ergogenic responses at higher altitudes.

2.4.3 Central and Peripheral Adaptations of the Native Highlander; Physiological Evidence for a Superior Athlete?

High altitude populations such as the Tarahumara Indians of North-Western Mexico and the Nandi tribe of Kenya have been noted for their remarkable endurance performances (Balke et al 1969; Fedders et al 1980). The apparently disproportionate running success of the native highlander is illustrated in Figure 2.6. This figure represents data obtained from athletes who were born and raised at a median altitude of 2000 m above sea-level. This phenomenon has prompted several comparative investigations into what, if any, physiological adaptations mediated by environmental hypoxia could contribute to their superiority in distance running events. Much interest has focused on the four steps of the oxygen transport system, namely: 1] pulmonary ventilation 2] pulmonary diffusion 3] circulatory oxygen transport and 4] tissue oxygen extraction.

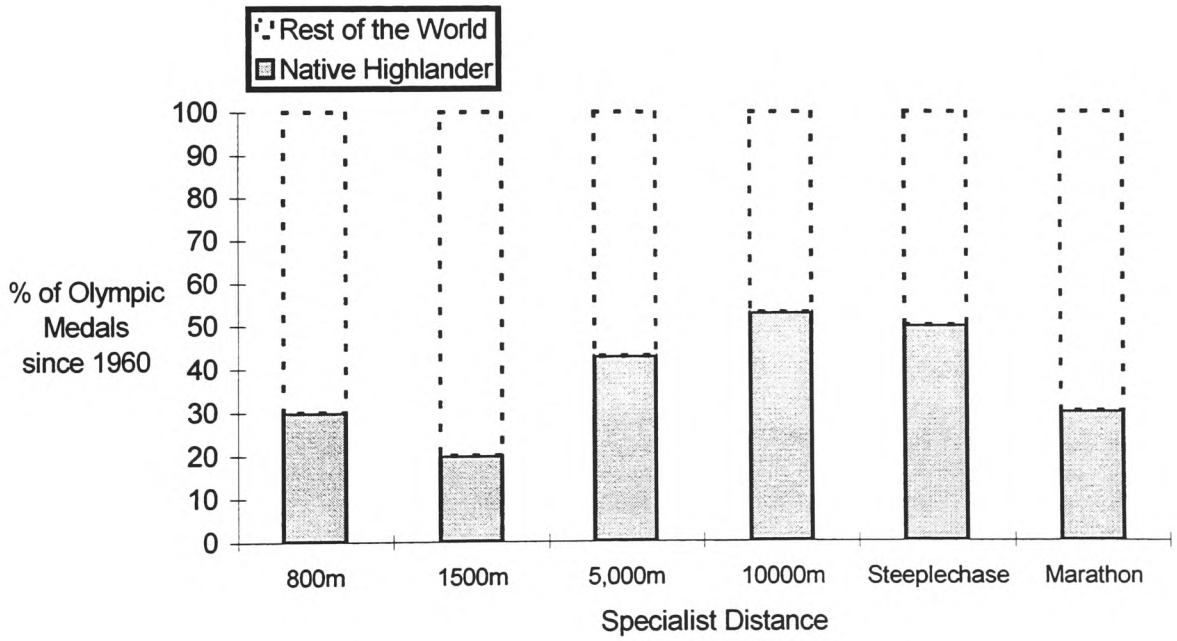


Figure 2.6 Distance Running Success of the Native Highlander (Born and Raised at a Median Altitude of 2,000 m)

To what extent environmental hypoxia contributes to the distance running success of the native highlander is presently unclear (Saltin, 1996). Scientific investigations have studied native Tibetans, Han "Chinese" and native lowlanders in an attempt to differentiate between the genetic and developmental bases of physiological adaptation. The Tibetan population has lived at altitude (1,500-4,700 m) for between 50,000 to 100,000 years

(Ward et al, 1995) which is longer than any other population on earth (Denell et al 1988). The Han Chinese have resided at a similar altitude for approximately 45 years.

2.4.3.1 Pulmonary Ventilation

Although equivocal, several investigations have reported lower values for the ventilatory equivalent for oxygen ($\dot{V}_E/\dot{V}O_2$) in native highlanders in comparison to lowlander controls during submaximal and maximal exercise (Kollias et al 1968; Frisancho et al 1973 and Zhuang et al 1993). A blunted hypoxic ventilatory response (HVR) and larger lung volumes (Sun et al 1990 and Zhuang et al 1993) have been implicated in this response.

The physiological implications of a decreased $\dot{V}_E/\dot{V}O_2$ for exercise performance are clear when one considers that, in the ventilation range of 90 to 130 L.min^{-1}, the oxygen consumption of the respiratory muscles ($\dot{V}O_2$rm) has been demonstrated to increase by 4.4 ml/min^{-1} per 1 L.min^{-1} increase in $\dot{V}_E$ (Shephard, 1966). Bye et al. (1984) have suggested that $\dot{V}O_2$rm may be as high as 1 L.min^{-1} min during maximum exercise in sedentary subjects. Despite the paucity of data on elite athletes, in particular distance runners who are capable of achieving $\dot{V}_E$ max's in excess of 200 L.min^{-1} (personal observations, British Olympic Medical Centre, London, UK), the mechanical costs of breathing are probably greater due to the exponential increase in $\dot{V}O_2$rm at such high $\dot{V}_E$ levels (Otis, 1954). Whilst $\dot{V}O_2$rm has not been quantified in the native highlander, it is tempting to speculate that a lower $\dot{V}_E/\dot{V}O_2$ would improve systemic oxygen supply to the active musculature.

2.4.3.2 Pulmonary Diffusion

Native highlanders have pulmonary diffusion capacities that are between 20-50% greater than those obtained in lowlander controls (DeGraff et al 1970 and Dempsey et al 1971). This has been attributed to an enlarged lung volume which results in an increased alveolar surface area and capillary blood volume (Ward et al, 1995).

Some elite athletes become hypoxaemic during intensive exercise at sea-level (Dempsey et al 1984 and Hammond et al 1986). This has been attributed to a diffusion impairment caused by a decreased pulmonary capillary transit time (PCTT) which prevents an equilibrium between end capillary PO_2 with the alveolar PO_2 (PAO_2) from being achieved (Dempsey et al 1984). This mechanism and the implications for exercise performance are discussed in more detail in Section 2.6.1. An increased capillary blood volume, typical of

the native highlander, would have the effect of increasing pulmonary capillary transit time, assuming that pulmonary blood flow at sea-level is similar to that observed in an elite native lowlander athlete:

$$PCTT = \frac{Vc\ (mls)}{Q\ (ml/s)}$$ Whipp and Ward (1994)

where:

PCTT - *mean pulmonary capillary transit time*
Vc - *capillary blood volume*
Q - *pulmonary blood flow*

This would attenuate the alveolar to arterial PO_2 difference ($[A\text{-}a]O_2$) and increase arterial oxygen saturation (SaO_2) thus improving exercise performance. The ergogenic benefits of attenuating arterial desaturation would be more pronounced at altitude due to the lower PAO_2. To the author's knowledge, comparative investigations of PCTT during maximal exercise performance in the elite native highlander at sea-level have not been investigated.

2.4.3.3 Circulatory Oxygen Transport

2.4.3.3.1 Erythropoietin (EPO) and Haemoglobin (Hb) Concentration

Winslow and Monge (1987) demonstrated that a native highlander's resting Hb concentration increased exponentially at altitude. The physiological mechanisms responsible for the maintenance of secondary polycythaemia at altitude are not fully understood, but recent evidence from Berglund et al. (1992) and Schmidt et al. (1993) would suggest that EPO is not a contributory factor. Thus, the elevated Hb concentrations may be the consequence of a haemoconcentration as plasma volume has been reported to be 27% lower in native highlanders (Sanchez et al 1970). The ergogenic benefits and physiological limitations of increasing Hb concentration in relation to exercise performance have been previously discussed in Section 2.4.2.

2.4.3.3.2 Blood Flow

Resting cardiac output ($\dot{Q}$) of the native highlander is similar to that of the acclimatised lowlander (Lenfant et al 1971). However, Vogel et al. (1974) have identified that the native highlander can maintain $\dot{Q}$ more effectively during maximal exercise in *acute*

hypoxia when compared with their lowlander counterparts. Maximum heart rate is less limited in chronic hypoxia and a decreased ß-sympathetic tone (Zhuang et al 1993) may increase blood flow due to an attenuation of sympathetically-mediated arterial vasoconstriction. This has been shown to increase $\dot{Q}$ following 10 days at sea-level, despite no significant change in total blood volume (Hartley et al 1967). Cerebral blood flow is also greater during physical exercise in the Tibetan population. Using non-invasive Doppler ultrasound, Huang et al. (1992) measured blood flow velocity in the internal carotid arteries of matched Tibetan (lifelong residence at 3,658 m) and Han "Chinese" (1-15 years residence at 3,658 m) subjects. The Tibetans attained higher mean values for flow velocity and cerebral oxygen delivery at workloads that elicited between 30 to 100% $\dot{V}O_{2max}$. Huang et al (1992) concluded that an increased cerebrovascular response is an important adaptation which serves to offset the decrease in SaO_2.

2.4.3.4 Peripheral Adaptations

Vogel et al. (1974) have demonstrated that native highlanders attain higher arteriovenous O_2 differences during maximal work when compared with native lowlander controls; evidence for an enhanced peripheral O_2 extraction. A rather limited number of investigations have characterised the biochemical and morphological structure of skeletal muscle. Although equivocal, the data would suggest that the skeletal muscles of the native highlander are characterised by a number of peripheral adaptations which facilitate the transfer of oxygen from the capillaries to the mitochondria. A critical examination of some of these adaptations and the possible implications for exercise performance is discussed below:

2.4.3.4.1 Myoglobin Concentration

At present, there is no convincing evidence to suggest that tissue myoglobin concentration is greater in the native highlander. An investigation by Reynafarje et al. (1962) demonstrated higher intramuscular myoglobin concentrations in native Peruvians resident at 4,400 m in comparison to values obtained from a sea-level control group (7.03 mg g^{-1} tissue vs 6.07 mg g^{-1} tissue). However, the authors failed to quantify the aerobic fitness levels of their subjects. The increased myoglobin concentrations may simply have been the consequence of an improved training status.

2.4.3.4.2 Muscle Enzymatic Composition and Substrate Utilisation

Saltin et al. (1995) compared muscle enzymatic compositions between elite Scandinavian and Kenyan distance runners. Whilst morphological and ultrastructural characteristics were similar, 3-hydroxy-acyl CoA dehydrogenase and lactate dehydrogenase$_{1\text{-}2}$ enzyme activities were greater in the Kenyan athletes (+24% and +27% respectively). The authors concluded that these enzymatic adapations were implicated in the lower blood lactate and increased lipid oxidation observed during exercise in the Kenyan athletes. In contrast, a decreased intracellular oxidative enzyme content has been observed in highlanders resident at higher altitudes of 3,000 to 4,500 metres. Decreased concentrations of malate dehydrogenase (Rosser et al 1993), citrate synthase and 3-hydroxyacyl-CoA dehydrogenase (Kayser et al 1996) appear to be the consequence of significant decreases (~4-28%) in total mitochondrial volume density (Hoppeler et al 1990; Howald et al 1990; MacDougall et al 1991 and Kayser et al 1991, 1992, 1996). However, a higher ratio between $\dot{V}O_2$ peak and mitochondrial volume density has been observed in the native highlander (Hochachka et al 1991; Matheson et al 1991 and Kayser et al 1991), which the authors have attributed to a tighter coupling between ATP hydrolysis and oxidative phosphorylation. Nuclear magnetic resonance imaging studies have identified lower concentrations of muscle adenylate and phosphogen in native highlanders during exercise; the consequence of which is a decrease in pyruvate flux to lactate (Hochachka et al 1992). It has been suggested that the mitochondrial function of the native highlander is different from that of the native lowlander (Kayser et al 1996). Whilst this possibility exists, a recent investigation identified that the adaptation of native Tibetans to high altitude were not caused by mitochondrial DNA mutations (Torroni et al 1994).

Glycolytic enzyme activities are greater in native highlanders. Rosser et al. (1993) investigated three Quechua natives in the Peruvian Andes (3,300 m) and three lowlanders (< 700 m). Despite the small sample size, they demonstrated that lactate dehydrogenase activity (LDH) was 28% greater ($P \leq 0.0001$) in Type I muscle fibres than that observed in lowlander controls. They concluded that chronic hypoxia produced a shift from oxidative to predominantly glycolytic metabolism in type I muscle fibres. A recent investigation by Kayser et al. (1996) did not identify any differences in L-lactate dehydrogenase and hexokinase activity when comparing second generation Tibetans at moderate altitude

(1,300 m) with Nepalese lowlander controls. The latter findings would suggest that the previous reports of increased glycolytic activity were invoked by hypoxia per se and were not just an inborn feature.

2.4.3.4.3 Ultrastructural Composition of Skeletal Muscle Fibres

Muscle fibre type distribution is comparable between native highlanders and lowlanders (Rosser et al 1993; Saltin, 1995 and Kayser et al 1996). However, Kayser et al. (1991) have identified smaller muscle fibres and a correspondingly greater relative capillary density in native highlanders. Whilst these findings are equivocal (Rosser et al 1993), it has been suggested that a shorter O_2 diffusion path between capillaries and mitochondria, in conjunction with a denser capillary bed serving a reduced mitochondrial volume may enhance peripheral oxygen conductance (Kayser et al 1996).

In contrast, two investigations have demonstrated that the native highlander is characterised by a lower lipid content (Kayser et al 1991 and Kayser et al 1996). The significance of this adaptation is not understood (Kayser et al 1996) but it may reduce intracellular O_2 flux. Oxygen is more soluble in nonpolar hydrocarbons than aqueous solution and thus a decreased intramuscular lipid content could potentially decrease the Krogh diffusion constant for O_2 denoted KO_2 (Krogh, 1919). Whilst this mechanism has been elucidated using fish skeletal muscle (Desauliniers et al 1996), the implications of a decreased intramuscular lipid content on KO_2 in human skeletal muscle has not been investigated (personal communication, Professor D.Jones, University of Birmingham, UK).

2.4.3.5 Anthropometrics

There is some evidence which suggests that the biomechanical running efficiency of the native highlander is superior due to their longer bone lengths and tendon insertions which would potentially facilitate endurance performance by decreasing the energy costs of locomotion (Himes, 1988). A greater storage of elastic energy in the Achilles and triceps surae tendons during the stretch-shortening cycle would effectively increase the efficiency of conversion of chemical energy into mechanical energy and thus improve running efficiency (Goldspink, 1977; Himes et al 1988 and Pate et al 1992). The anatomical differences between the native highlander and lowlander require further investigation.

A summary of the major physiological adaptations that are characteristic of the native highlander are outlined in Table 2.2. The significance of these, and possibly other as yet unknown adaptations have been elucidated in a series of investigations. Although equivocal, the native highlander can attain higher values for $\dot{V}O_{2max}$ (Sun et al 1990; Curran-Everett et al 1992 and Zhuang et al 1993) power output (Ge et al 1994) and arterial oxygen saturation (Favier et al 1995) during maximal exercise and decreased oxygen uptake expressed relative to body mass$^{-0.75}$ (Saltin et al 1995), ammonia concentrations (Svedenhag et al 1991 and Saltin et al 1995) and blood lactate (Ge et al 1994; Hochachka et al 1991 and Saltin et al 1995) for a given submaximal workload in comparison to the native lowlander both at sea-level and at altitude. Raynaud et al. (1974) and Fellmann et al. (1988) have demonstrated a more rapid disappearance of blood lactate in native highlanders who were born and living in La Paz, Bolivia (~3,700 m). The latter investigators demonstrated that the $t_{1/2}$ of blood lactate in native highlanders was 8.8 minutes whereas it was significantly greater (14.4 minutes, $P < 0.01$) in a matched control group based at sea-level (Fellmann et al 1988). In contrast, the native highlander does not appear to possess an augmented anaerobic capacity (Bedu et al 1994 and Falgairette et al 1994).

Table 2.2 Physiological Adaptations of the Native Highlander

Positive adaptation	Author (Year)	Positive adaptation	Author (Year)
↑ myoglobin content	Reynarfarje (1962)	↓ capillary to	Kayser (1991)
↓ HVR/↓ exercise	Milledge (1967)	mitochondrion	
ventilation/↓$\dot{V}O_2$rm		diffusion distance	
↑ lung diffusion	Dempsey (1971)	↓ hypoxic pulmonary	Groves (1993)
↑ $\dot{Q}$ during maximal	Vogel (1974)	vasoconstriction	
exercise		↓ ß sympathetic/	Zhuang (1993)
↑ Hb concentration	Winslow (1987)	↑ parasympathetic	
↑ vital capacity	Sun (1990)	tone during exercise	
↑ capillary density	Kayser (1991)	↑ glycolytic enzyme	Rosser (1993)
↑ cerebral O_2 delivery	Huang (1992)	capacity	

HVR - hypoxic ventilatory response
$\dot{V}O_2$rm - respiratory muscle oxygen uptake
↑ - increase
Hb - haemoglobin
$\dot{Q}$ - cardiac output
↓ - decrease

2.4.3.6 Physiological Adaptations of the Native Highlander; Genetic, Behavioural or Environmental?

To what extent these physiological adaptations are acquired due to inheritance, training or environmental hypoxia is not well defined. Frisancho et al. (1995) conducted $\dot{V}O_{2max}$ tests on 268 native highlanders who were divided into separate cohorts according to occupational activity level and duration of residence at altitude. They identified that $\dot{V}O_{2max}$ was inversely related to age of arrival at altitude. Whilst subjects with a higher occupational activity level achieved higher $\dot{V}O_{2max}$ values, it was clear that even high levels of physical activity among subjects acclimatised to high altitude during adulthood was not sufficient to equal the magnitude of adaptation acquired during a lifetime of exposure. Using linear regression analyses, the authors quantified the components that contributed to the variability of $\dot{V}O_{2max}$ at high altitude. They concluded that 20-25% of the variability was related to a developmental component (age of arrival at altitude), 20-30% to a genetic component and the majority of the variance (45-60%) was related to an environmental component (occupational activity level). In the first investigation of its kind to accurately quantify training loads in a cross section of untrained to World class Kenyan and Scandinavian subjects, Saltin et al. (1995) have also suggested that the major reason for the

native highlander's running success is determined by the unusually high training loads that are conducted at an early age. From the age of 7 years, most children in western Kenya were reported to cover up to 25 km a day; whilst junior athletes aged 15 years completed 100 km a day at high intensity equivalent to 80 to 90% $\dot{V}O_{2max}$ (Saltin et al 1995).

The influence of genetic factors on quantitative oxygen transport was recently investigated in a unique study by Beall et al. (1994). They identified a major gene which enhances arterial oxygen saturation in sedentary Tibetan natives. The physiological significance of this was demonstrated by Niermeyer et al. (1995) who concluded that genetic adaptations to hypobaric hypoxia resulted in improved oxygenation and conferred resistance to subacute infantile mountain sickness. These adaptations were more pronounced in a cohort of Tibetan newborns in comparison to Han newborns.

In summary, although it would appear that continued exposure to environmental hypoxia contributes to the physiological superiority of the native highlander, it is not the only factor. In addition, a lifetime or perhaps generations of altitude exposure are responsible for the biological distinctiveness of this population. Whether the native lowlander can experience similar physiological adaptations during an acute sojourn to altitude and the subsequent implications for physical performance will be discussed in the following sections.

2.5 PHYSIOLOGICAL ACCLIMATISATION TO ALTITUDE AND RESPONSES FOLLOWING RETURN TO SEA-LEVEL

A number of physiological adaptations are invoked during acute and chronic exposure to environmental hypoxia which strive to maintain adequate tissue oxygenation. Unfortunately an operational term for either "acute" or "chronic" does not exist in the literature (Fulco and Cymerman, 1988) which tends to lead to inconclusive and contradictory conclusions. However, in the present context, acute is defined as exposure to hypoxia for 24 h or less. whereas chronic is defined as >24 h to years.

Connett et al. (1990) have summarised the interaction between the major metabolic subsystems that "defend" intracellular PO_2 despite the challenge imposed by a decreased inspiratory PO_2 (P_IO_2). The physiological mechanisms responsible for these adaptive changes in convective and diffusive transport and subsequent implications for exercise performance will be examined in the following sections. The interactive effects of physical exercise on the rate of acclimation/acclimatisation will also be discussed.

2.5.1 Respiratory Acclimatisation

2.5.1.1 Pulmonary Ventilation and Acid-Base Balance

An increase in pulmonary ventilation ($\dot{V}_E$) is one of the fundamental physiological responses that occurs during the initial stages of acclimatisation to environmental hypoxia. Hypoxia and carbon dioxide (CO_2) stimulate $\dot{V}_E$ causing an increase in the arterial partial pressure of oxygen (PaO_2) and a decrease in the arterial partial pressure of carbon dioxide ($PaCO_2$). Whilst some of these respiratory adaptations at altitude benefit exercise performance, others may be considered less beneficial.

2.5.1.1.1 At Altitude

Acute

On acute exposure to altitude, $\dot{V}_E$ remains unchanged unless the P_IO_2 decreases to approximately 100 mmHg, equivalent to an altitude of 3000 m. (Rahn and Otis, 1949). At and beyond this level of hypoxia, pulmonary ventilation increases hyperbolically, a response known as the Hypoxic Ventilatory Response (HVR) which is mediated by individual

hypoxic chemosensitivity (Schoene, 1982). The purpose of the HVR is to increase alveolar PO_2 (PAO_2) by primarily increasing respiratory frequency (Schoene, 1982).

The sensitivity of the HVR and subsequent implications for exercise performance at altitude are poorly understood (Ward et al, 1995). A brisk HVR has been associated with an impaired mental capacity (Hornbein et al 1989) and accentuates the hypoxia associated with periodic breathing during sleep at high altitude (Lahiri et al 1984). However, other investigators consider a brisk HVR to be advantageous for exercise performance at moderate altitudes due to a leftward shift in the oxyhaemoglobin disassociation curve, an increase in arterial oxygen saturation (SaO_2) and subsequent increase in PaO_2 (Schoene et al 1984). It has also been suggested that a brisk HVR may protect against acute mountain sickness (Richalet et al 1988; Hackett et al 1988 and Masuda et al 1992); a condition which in most cases is benign, but may progress to the life threatening form of high altitude pulmonary and cerebral oedema (Milledge, 1994).

Chronic

Following chronic exposure to environmental hypoxia, $\dot{V}_E$, in response to any decrease in P_IO_2, is elevated above sea-level values. Figure 2.7 summarises the increases in $\dot{V}_E/\dot{V}O_2$ at a variety of altitudes.

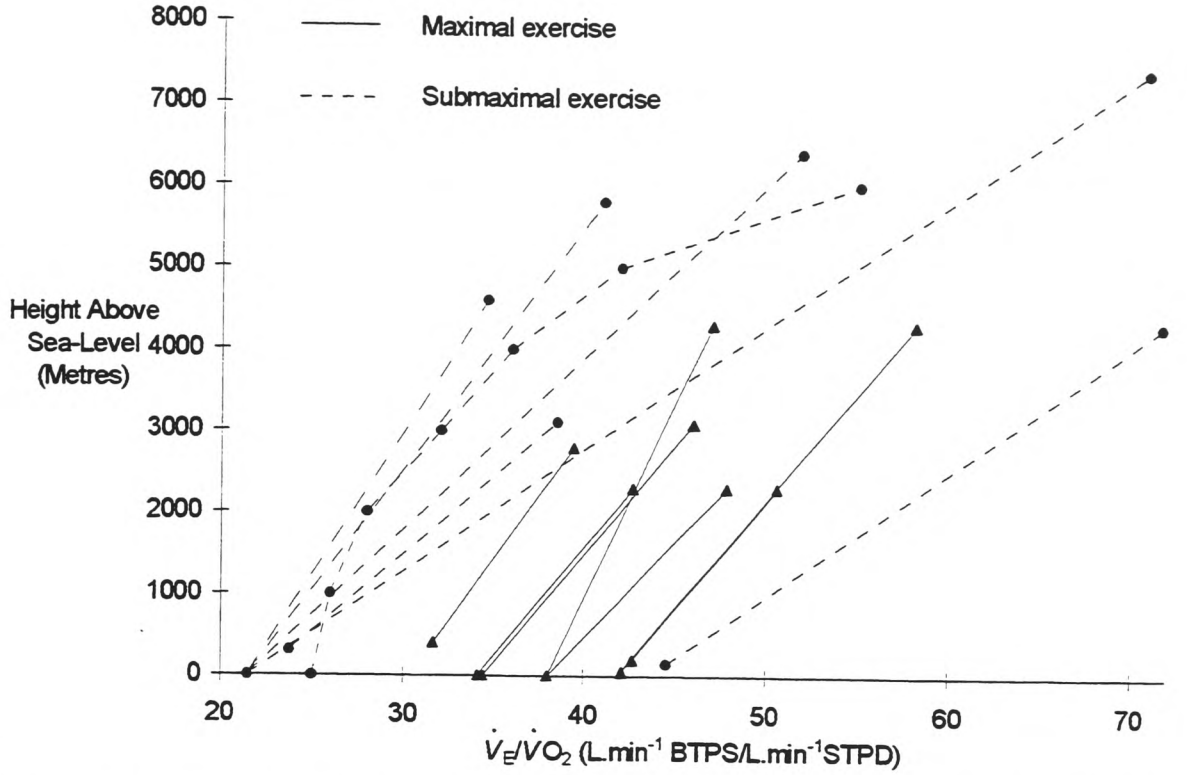


Figure 2.7 Chronic Changes in Ventilatory Equivalent for Oxygen ($\dot{V}_E/\dot{V}O_2$) at Altitude

Values are Means
Data based on: Pugh (1964), Balke et al. (1965), Reeves et al. (1967), Faulkner et al. (1967, 1968), Saltin et al. (1968), Daniels et al. (1970), Dill et al. (1971)

Further increases in $\dot{V}_E$ are mediated by changes in the ventilatory response to CO_2, termed the hypercapneic ventilatory response (HCVR). For a full discussion of the physiological mechanisms that are implicated in the resetting of the HCVR at altitude, see Ward et al (1995). PaO_2 is subsequently increased and $PaCO_2$ decreases which results in an increase in blood pH in accordance with the Henderson-Hasselbalch equation:

$$pH_a = pK' + \log \frac{[HCO_3^-]_a}{\alpha PaCO_2}$$

where:

pK'	- ionisation constant of carbonic acid (6.1)
$[HCO_3^-]_a$	- arterial bicarbonate concentration
α	- solubility coefficient for CO_2 in plasma (0.03 mmol/mmHg at 37°C)
$PaCO_2$	- arterial PCO_2

A decreased pH in the renal tubular cells stimulates the release of HCO_3^- ions from the kidneys; a process termed metabolic compensation for respiratory alkalosis. Whilst this restores the "normal" HCO_3^- : PCO_2 ratio, there are other potentially less favourable implications for exercise performance. Cerretelli and DiPrampero (1985) and Maibaurl et al. (1987) have identified that at any given blood lactate concentration, the blood pH was lower during exercise at moderate to high altitudes in comparison to normoxia. A decrease in extracellular pH also decreases the efflux of muscle lactate into the blood due to a decrease in the activity of the sarcolemmal lactate transporter (Roth and Brooks, 1990). Thus, a disparity between muscle and blood lactate has been noted during exercise at altitude; McLellan et al. (1990) demonstrated that the concentration of lactate in the muscle was 2.2 times greater during supramaximal exercise than that in the blood following acute exposure to 4,800 m. Prolonged exercise above the anaerobic threshold may therefore decrease the athlete's ability to tolerate lactate accumulation. Shephard (1992) has suggested that a decrease in blood and tissue bicarbonate concentrations will affect fluid balance. This may be implicated in a progressive decrease in stroke volume and corresponding cardiac output which have been observed in subjects at rest and during exercise at altitude (Wolfel et al 1991).

2.5.1.1.2 Following Return to Sea-Level

Several investigators have demonstrated that endurance athletes are characterised by a blunted HVR (Byrne-Quinn et al 1971 and Bjuerstrom and Schoene, 1986), the significance of which is not fully understood. It has been suggested that this reduces the ventilatory cost of breathing at sea-level (Wolski et al 1996) which has been estimated at 30% $\dot{V}O_{2max}$ during maximal exercise (Levitzky, 1995). Two separate investigations have demonstrated that 3 to 5 weeks of intermittent hypoxic training at an ambient PO_2 equivalent to 2500 to 5700 m significantly increased the HVR in sedentary subjects (Levine et al 1992 and Benoit et al 1992). If the HVR induced by altitude training overides the blunted HVR at sea-level in the *elite athlete*, this would present a clear disadvantage as it would increase the ventilatory cost of breathing and decrease exercise performance following return to sea-level.

2.5.1.2 Ventilation/Perfusion (V_A/Q_C) Relationships

Pulmonary vascular resistance and pressure increases on acute and chronic exposure to hypoxia. Resting pulmonary artery pressure has been shown to increase by 18% at

2,370 m, 60% at 6,100 m, 226% at 7,620 m and 220% on the summit of Mt Everest at ~ 8,840 m and is more pronounced during physical exercise (Sime et al 1974 and Reeves et al 1987). It has been suggested that elevated pulmonary vascular resistance is a maladaptive response to altitude which increases the workload on the right ventricle and has little effect on attenuating ventilation-perfusion ($\dot{V}_A/\dot{Q}_C$) mismatch (Groves et al 1993). This response has also been implicated in the genesis of the more life threatening condition of pulmonary oedema (Hultgren, 1978).

2.5.1.3 Pulmonary Diffusion

Despite significant increases in the ventilatory equivalent for oxygen ($\dot{V}_E/\dot{V}O_2$)at altitude (Figure 2.7.) arterial oxygen saturation (SaO_2) decreases at rest and during exercise (Levine et al 1992; Favier et al 1995 and Vallier et al 1996). Whilst part of this response can be attributed to a ventilation perfusion ($\dot{V}_A/\dot{Q}_c$) mismatch caused by an increase in pulmonary hypertension, a diffusion limitation of oxygen transfer predominates whic results in a widening of the alveolar-arterial PO_2 gradient (Houston et al 1987 and Groves et al 1987). Despite the prevailing hypoxaemia that develops, the diffusing capacity of the alveolar capillary membrane itself does not change at altitude (West, 1962).

2.5.2 Circulatory Oxygen Transport

2.5.2.1 Erythropoietin (EPO) and Haemoglobin (Hb) Concentration

2.5.2.1.1 At altitude

Acute

There is an initial increase in resting Hb concentration at altitude due to rapid decreases in intra and extracellular water content and plasma volume (Wolfel et al 1991 and Kayser, 1994). Whilst physical exercise may attenuate a sodium and water diuresis due to activation of the renin-angiotensin-aldosterone system, (Milledge, 1992) overall fluid loss from these compartments in active subjects at altitude has been estimated at 1 to 2 L (Kayser, 1994). Normalisation of the plasma volume may take as long as 2 months at 4,500 m (Reynafarje et al 1959). As a result of these fluid shifts, there is a tendency towards a decreased total blood volume at altitude. Few studies have quantified these changes, but a recent investigation by Wolfel et al. (1991) has demonstrated that acute

ascent to 4,300 m resulted in a 11% decrease in plasma volume and a 7% decrease in total blood volume.

To counteract the decrease in PaO_2 at altitude, hypoxia *per se* activates the release of erythropoietin (EPO) from the kidney, a glycoprotein hormone which stimulates erythrocytic precursor cells (Schmidt et al 1993). Significant advances in the understanding of the kinetics of EPO release at altitude have been made possible since the introduction of the radio-immunoassay technique developed by Nielsen (1988). This has improved EPO detection limits from 50 $mUml^{-1}$ to <10 $mUml^{-1}$. However, the precise mechanism by which EPO producing cells perceive oxygen is not completely understood (Mairbaurl, 1994). Adenosine has been powerfully implicated in the activation of receptors on the cell membrane of EPO producing cells which increase stimulatory proteins that result in the subsequent biosynthesis of EPO (Krantz et al 1991). Other modulators such as H^+, pCO_2, lactate, prostaglandins, β-adrenergic stimulators, vasopressin and anabolic steroids may also stimulate EPO production (Berglund, 1992). It has also been suggested that the additive stimulus of exercise compounds the normal EPO release during a hypoxic exposure through increased stimulation of thyroid and supra-adrenal hormones or changes in splanchnic blood flow (Jelkman et al 1986; Schmidt, 1990 and Berglund, 1992). This would explain the higher haematocrit values that have been reported in altitude acclimatised endurance athletes when compared with altitude acclimatised sedentary controls (Berglund et al 1992).

Chronic

Chronic exposure to environmental hypoxia causes a further increase in resting EPO and maximal concentrations have been reported following 2 to 3 days at altitude (Berglund, 1992). Thereafter, EPO decreases to a steady state level which is slighlty elevated above pre-altitude concentrations. Klausen et al. (1991) demonstrated a 31% increase in resting EPO ($P < 0.05$) following 3 days of altitude training at 1,695 to 2,300 m in 6 elite cross country skiers. These values decreased by day 7 at altitude, but were still 14% ($P < 0.05$) greater than pre-altitude concentrations. The significance of this decrease in EPO during chronic altitude training is not understood and is at present the subject of much interest. However, it has been suggested that either a downregulation of the renal oxygen sensing mechanism or an increased flow of oxygen to EPO producing cells may be implicated in this response (Mairbaurl, 1994). Thus, the intensity and duration of the hypoxic stimulus,

physical exercise and a range of metabolic factors contribute to the variability of EPO increase at altitude (Figure 2.8). In one study, the individual increase in resting EPO ranged from 3 to 134-fold (Richalet et al 1993).

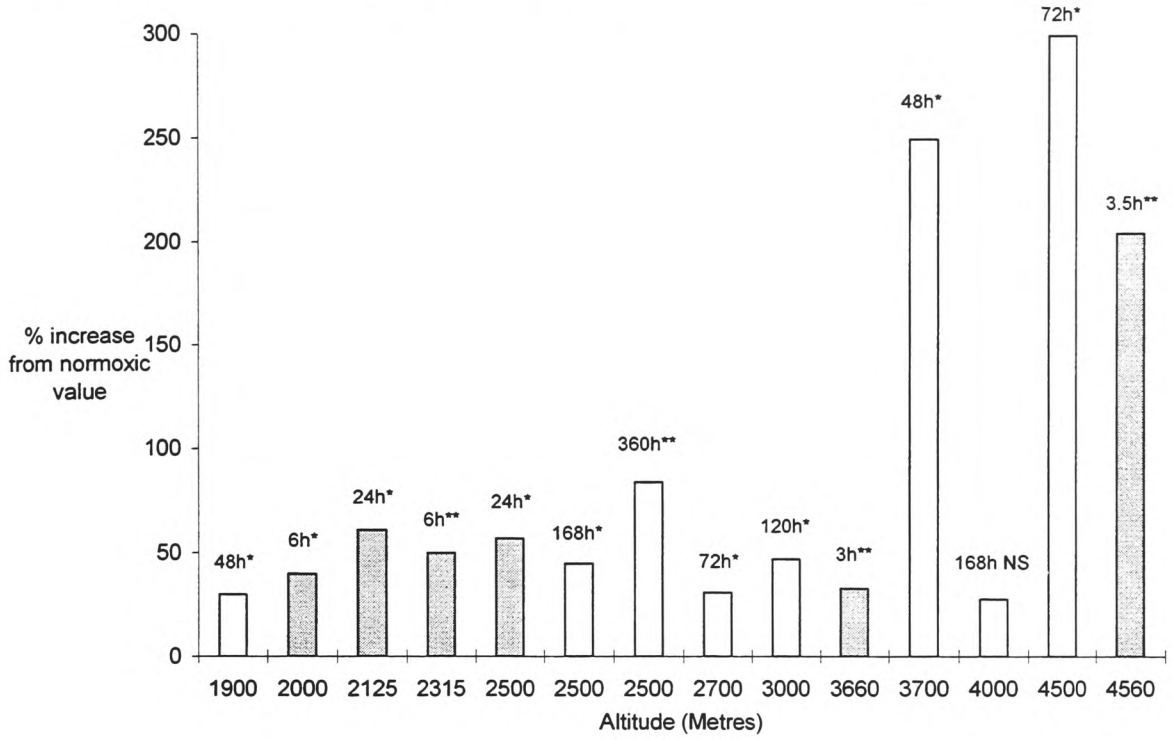


Figure 2.8 Time Course and Changes in Resting Erythropoietin (EPO) Concentration During Acute and Chronic Hypoxia

Values are Means
Shaded bars represent acute hypoxia (< 24 h)
Clear bars represent chronic hypoxia (>24 h)
* - Significant difference from normoxic value ($P < 0.05$)
** - Significant difference from normoxic value ($P < 0.01$)

Data based on: Milledge and Cotes (1985), Eckardt et al. (1989), Winslow et al. (1989), Klausen et al. (1991), Schmidt et al. (1991), Berglund (1992), Knaupp et al. (1992), Quick et al. (1992), Laitinen et al. (1994), Mattila et al. (1994), Puranen et al. (1994), Stray-Gundersen et al. (1994), Vallier et al. (1996) and Stray-Gundersen et al. (1997).

An increased secretion of EPO results in an elevated reticulocytosis and Hb concentration increases independently of a haemoconcentration. Following 18 days at 4,300 m, Wolfel et al. (1991) demonstrated that erythrocyte volume increased from 2,399 ml on acute ascent to 2,877 m ($P < 0.05$). However, erythrocyte volume does not increase until about 4 days at altitude (Klausen et al 1991) and maximum reticulocytosis occurs after 8 to 10 days at altitude (Hartmann et al 1990). Thus, the impressive increases in Hb concentration reported by investigators during the early phases of altitude acclimatisation are probably

the result of a haemoconcentration (Klausen et al 1991 and Ingjer et al 1992). In a review, Berglund (1992) stated that "true" Hb concentrations increased at a rate of 1% per week in lowlander athletes who trained at altitudes ranging between 1829 to 3048 m. Assuming a resting Hb concentration of 15g/dl and a total blood volume of 7000 ml in elite male distance runners (personal observations, British Olympic Medical Centre), total haemoglobin values would increase at a rate of 10.5 g per week. The time course for complete haematological adaptation would occur when the native lowlander has similar Hb concentrations to the native highlander. The necessary adaptation time at 2,500 m has been estimated at 12 weeks (Berglund, 1992).

2.5.2.1.2 Following Return to Sea-Level

In a single group experimental design, Stray-Gundersen et al. (1995) demonstrated that following 4 weeks of altitude training at 2,500 m in 13 collegiate runners, plasma volume decreased from a pre-altitude mean of 59 ml/kg to 54.7 ml/kg after 1 wk at sea-level ($P < 0.05$). These findings have been supported by other investigators who have reported an 8% decrease in plasma volume that is maintained for between 1 to 3 weeks post altitude (Dill et al 1974 and Levine et al 1992). Thus, the increased Hb concentrations that have been reported following 1 to 7 days return to sea-level (Ingjer et al 1992; Klausen et al 1991 and Laitinen et al 1995) may reflect a haemoconcentration as rates of EPO secretion remain unchanged or even decrease post altitude (Milledge et al 1985 and Klausen et al 1991). Much of the experimental investigations in the research literature have not quantified these important haematological changes.

Whilst an acute stay at sea-level depresses plasma volume, there is some evidence which suggests that chronic exposure to sea-level normoxia following altitude training may result in a plasma volume expansion and cause pseudoanaemia. Haymes et al. (1986) demonstrated a decrease in Hb concentrations after 1 to 2 months at sea-level. Reynafarje et al. (1959) also demonstrated decreases in a group of high altitude residents native to 4,300 m after 2 to 4 months at sea-level. Whilst the precise physiological mechanisms responsible for the reduced Hb concentration is unknown, the pseudoanaemia may be a reflection of expanded plasma volumes.

2.5.2.2 Erythrocyte 2,3-Diphosphoglycerate (2,3-DPG)

Whilst 2,3-DPG is not advantageous for O_2 loading in the lungs due to a rightward shift of the oxygen dissociation curve (Ward et al 1995), it allows sufficient O_2 to be released from Hb to the tissues despite the initial respiratory alkalosis (Mairbaurl, 1994). It has been estimated that the standard P_{50} (PO_2 for 50% saturation of Hb with oxygen) increases in the range of 0.5 to 2 mmHg per μM/gHb of 2,3-DPG (Mairbaurl et al 1994).

Initially, concentrations of erythrocyte 2,3-DPG have been shown to increase on acute exposure to altitude (Sutton et al 1988; Samaja et al 1993 and Puranen et al 1994). Further increases in 2,3-DPG occur according to the magnitude and duration of the hypoxic stimulus due to an EPO stimulated increase in reticulocyte count (Mairbaurl et al 1990). Exercise at altitude in the untrained state has been demonstrated to potentiate the increase in 2,3-DPG, whereas athletes with already elevated levels of 2,3-DPG at sea-level do not experience increases on ascent to moderate altitude (Mairbaurl et al 1987). However, these findings were recently questioned by Laitinen et al. (1995). They demonstrated that following 15 days of intermittent exposure to normobaric hypoxia (~2,500 m) 2,3-DPG concentrations increased by 15% ($P < 0.05$) in 7 trained athletes. On descent to sea-level, 2,3-DPG concentrations and P_{50} decrease immediately (Mairbaurl, 1994).

2.5.2.3 Blood Flow and Cardiac Function

2.5.2.3.1 At Altitude

Acute

Cardiac output ($\dot{Q}$) at rest and during submaximal exercise increases immediately on ascent to altitude (Hansen et al 1967; Saltin et al 1968 and Wagner et al 1986). This can be attributed to a marked increase in heart rate (HR) without any significant changes in stroke volume [(SV), Stenberg et al 1966]. The increase in submaximal HR occurs in response to a marked increase in ß-sympathetic activity which is time and altitude dependent (Zhuang et al 1993a). This is reflected by a rise in plasma and urinary catecholamine concentrations at altitude (Neureither et al 1996).

Chronic

In contrast, $\dot{Q}$ during physical exercise decreases during a prolonged exposure to altitude. Saltin et al. (1968) identified a 22% decrease in maximal $\dot{Q}$ measured by a dye-dilution technique following 2 wk at 4,300 m. Greater decreases have been demonstrated at higher altitudes; Pugh (1964) identified a 29% decrease in maximal $\dot{Q}$ determined using the acetyline rebreathing technique (Christensen, 1931) at 5,800 m in 4 subjects. These decreases have been attributed to a decrease in maximal heart rate and stroke volume with the latter resulting in lower right atrial pressure and smaller intraventricular volumes (Alexander et al 1983). Alleviation of hypoxia at altitude by the administration of a hyperoxic gas mixture has been shown to attenuate the reduction in maximal $\dot{Q}$ (Pugh, 1964 and Saltin et al 1968). However, whilst hyperoxia has variable effects on exercise heart rate causing either an increase (Pugh, 1964 and Saltin et al 1968) or a decrease (Reeves et al 1987), stroke volume for a given pulmonary wedge or filling pressure is not altered (Reeves et al 1987). This would suggest that the decrease in stroke volume is not caused by hypoxic depression of myocardial contractility and is probably related to a hypovolaemia due to a decreased plasma volume.

Despite the decrease in maximal $\dot{Q}$, the relationship between submaximal $\dot{V}O_2$ and $\dot{Q}$ is comparable to that observed at sea-level even on the summit of Mt Everest (Reeves et al 1987). Why maximal $\dot{Q}$ is not increased at altitude to augment systemic O_2 transport is not fully understood (Ward et al 1995). A recent review by Saltin (1996) speculated that this mechanism serves to optimise $\dot{V}_A/\dot{Q}_C$. An increase in pulmonary blood flow at altitude would result in a decreased pulmonary capillary transit time (PCTT) and a diffusion limitation would result in a more pronounced decrease in arterial oxygen content. The larger $\dot{Q}$ and an increased PCTT may explain why a decreased maximal heart rate has been observed in elite athletes when they train at even moderate altitudes equivalent to 2000 m (personal communication, Professor B.Saltin, University of Copenhagen, Denmark).

2.5.2.3.2 Following Return to Sea-Level

A limited number of investigations have documented the changes in $\dot{Q}$ following descent to sea-level. Balke et al. (1965) speculated that maximal $\dot{Q}$ would increase on return to sea-level due to a 16% increase in total blood volume following a 10 day sojourn to 2,300 m. However, it is becoming increasingly clear that arterial O_2 content (CaO_2) and not blood

volume is the major determinant of blood flow during physical exercise (Hartley et al 1967 and Wolfel et al 1991).

Using the dye-dilution technique (Vogel et al 1967), Hansen et al. (1967) reported a decrease in resting and exercise $\dot{Q}$ in comparison to pre-altitude measurements following return to sea-level after 15-18 days of training at 4,300 m. However, the authors did not identify if these changes were significant. Using a modified rebreathing technique (Cerretelli et al 1970), Boutellier et al. (1984) identified that following a 3 month expedition to 8,398 m, $\dot{Q}$ at 150W was 18% lower than pre-altitude values ($P < 0.01$).

2.5.3 Peripheral Adaptations

Whilst it would appear that total oxygen delivery to active skeletal muscle, a product of flow $\dot{Q}$ or limb blood flow determined by the thermodilution technique (Bender et al 1988 and Sullivan et al 1987)] and CaO_2 does not change during altitude acclimatisation (Wolfel et al 1991), is there any evidence to suggest that peripheral adaptations facilitate an improved diffusion of O_2 from the capillaries to the mitochondria?

2.5.3.1 Ultrastructural Composition of Skeletal Muscle Fibres

The effects of hypoxia per se on the ultrastructural composition of human skeletal muscle fibres is not well defined. Few investigations have incorporated a normoxically trained control group in the experimental design. Increases in physical activity during an altitude sojourn may also invoke adaptive changes in the peripheral characteristics of skeletal muscle (Simoneau, 1995) that are independent of hypobaric hypoxia.

2.5.3.2 Muscle Fibre Size and Capillary Density; Implications for Systemic O_2 Transport and Muscle Force

Capillary density expressed as the number of capillaries per mm^2 of skeletal muscle tissue increases during continuous altitude exposure due predominantly to a reduction in mean muscle fibre size (Table 2.3). Whilst this may facilitate O_2 diffusion due to a reduction in intercapillary distance (Ward et al 1995) and an increase in mean transit time for a given blood flow (Saltin, 1996), it would also decrease muscle force due to a decrease in cross sectional area (Saltin, 1983). A recent study by Narici et al. (1995) demonstrated that the potential for human skeletal muscle hypertrophy was lower in conditions of chronic

hypobaric hypoxia. They showed that isometric maximal voluntary contraction and the cross sectional area of the elbow flexors following 4 weeks of strength training at 5,050 m was significantly lower ($P < 0.05$) than that resulting from a matched training programme conducted in normoxia. Although atrophy is more pronounced at extreme altitudes (Rose et al 1988), it can also occur following prolonged exposure to 2100 m (Table 2.3). The decrease in muscle fibre size and increased capillary density has been demonstrated to persist for up to several months following return to sea-level (Oelz et al 1986).

Table 2.3 Skeletal Muscle Fibre Size and Capillary to Fibre Ratio Before and After Altitude Training

Author	Altitude	Duration	Fibre size $\mu m^2 10^3$		Capillary:fibre	
(Year)	(Metres)	(Days)	PRE	POST	PRE	POST
Continuous exposure						
Saltin '83	3700	168	4.8	4.2	1.3	1.2
Mizuno '90	2100-2700	14	5.8	5.4†	2.6	2.7
Svedenhag '91	2000	14	4.9	4.8	2.7	2.7
Svedenhag*	2100	28	5.9	5.5	2.8	2.7
Green '92	4300	21	6.1	6.3	0.8	0.9
Saltin '96	84	5.8	4.7	2.1	2.1	2.2
Intermittent exposure						
Terrados '88	2300	21-28	No data	No data	3.2	3.7
Terrados '90	2300	28	5.5	5.7	1.7	1.8
Desplanches'93	4100-5700	21	4.3	4.7†	1.9	2.2†

† - significantly different from PRE value ($P < 0.05$)
* - unpublished data (cited by Saltin, 1996)

Much interest has focused on the catabolic effects of altitude (Kayser, 1994). Physical inactivity (Levine et al 1992), malabsorption of nutrients (Westerterp et al 1994) and a depression of protein synthesis (Rennie et al 1983) via hormonal changes or a direct effect of hypoxia *per se* have been implicated in muscle wasting during continuous exposure to altitude. The fact that muscle fibre sub types remain unchanged at altitude (Table 2.4) would suggest that the catabolic effects of chronic hypoxia are not selective to specific myosin heavy chain isoforms.

Table 2.4 Percent Changes in Skeletal Muscle Fibre Sub Types Before (B) and After (A) Altitude Training

Author	Altitude	Duration	Type I		Type IIa		Type IIb		Type IIc	
(Year)	(Metres)	(Days)	B	A	B	A	B	A	B	A
Continuous exposure										
Mizuno '90	2100-2700	14	55	53	44	45	1	2	ND	ND
Green '92	4300	21	55	43	36	44	9	13	ND	ND
Intermittent exposure										
Terrados '88	2300	21-28	64	63	30	29	6	8	1	0.3
Terrados '90	2300	28	43	45	37	39	18	15	2	1

ND - No data

However, the peripheral responses to intermittent hypoxia appear to be different. Desplanches et al. (1993) have provided powerful evidence for capillary neoformation in response to intermittent hypoxic training. The authors reported a 13% increase in capillary to fibre ratio ($P < 0.05$) in conjunction with a 10% ($P < 0.05$) increase in muscle fibre size. Hoppeler (1997) recently advocated the use of intermittent hypoxic training as an ergogenic aid for both endurance and strength training. Current research is focusing on the potential role of hypoxia inducible factor 1 (HIF-1) as a modulator of muscle phenotype (Semenza et al 1994).

2.5.3.3 Muscle Myoglobin and Enzymatic Concentrations

Terrados et al. (1988) studied the effects of 3 to 4 weeks of either intermittent hypoxic or normoxic training on skeletal muscle enzyme activities and subsequent implications for endurance performance in 8 highly trained cyclists (Table 2.5). Four subjects trained in a hypobaric chamber at an ambient PO_2 equivalent to 2,300 m and the other 4 trained at sea-level. Whilst hypoxic training did not alter oxidative enzyme activities, muscle phosphofructokinase and lactate dehydrogenase activities decreased in the altitude trained group only ($P < 0.05$ vs pre-training value). As a consequence, blood lactate concentrations decreased and work capacity increased. However, data were analysed using parametric statistical analyses despite the fact that only 4 subjects were employed in each

group. It is unlikely that the data were normally distributed and it is quite possible that non significant results would have been obtained if the data were analysed using non-parametric ranking analyses.

In a unique follow up study, 10 subjects trained one leg under normoxic conditions and the contralateral leg was trained at an identical absolute work intensity under hypobaric conditions (~2,300 m) for 30 minutes a day, 4 times per week during a 4 week experimental period (Terrados et al 1990). In the only study to date using human subjects, the authors demonstrated that intermittent hypoxic training increased myoglobin content by 8% ($P < 0.05$). Citrate synthase activity also increased by 29% ($P < 0.001$) in the vastus lateralis muscle. As a result, exercise time to exhaustion increased 21% more in the hypobarically versus the normobarically trained leg ($P < 0.05$). The authors concluded that hypoxia *per se* and not substrate flux was the major stimulus for metabolic adaptation. The aetiology of these adaptations remains controversial, but there is evidence which suggests that the hypoxia-induced increases in catecholamine levels (Escourrou et al 1984 and Neureither et al 1996) increases β-Receptor stimulation via adenosine 3',5'-cyclic monophosphate which has been demonstrated to increase the synthesis rate of mitochondrial enzymes (Ji et al 1986).

In contrast, continuous exposure at higher altitudes has been shown to decrease oxidative enzyme capacities (Table 2.5) due to a reduction in mitochondrial volume density (Hoppeler et al 1990 and Howald et al 1990). However, a common trend during both intermittent and continuous hypoxic exposure is the reduction in glycolytic enzyme activity. This has been observed even at moderate altitudes of 2,300 m (Terrados et al 1988, 1990) and not just as a consequence of exposure to extreme hypoxia as was originally suggested by Green et al. (1989) and Howald et al. (1990). The physiological mechanisms responsible for a decreased glycolytic capacity are not understood (Green et al 1992). However, it is conceivable that a decrease in absolute training intensity is a contributory factor.

Table 2.5 Intramuscular Myoglobin and Bicarbonate Concentration and Maximal Enzyme Activities after Hypoxic Training in Native Lowlanders

Author (Year)	Altitude (Metres)	Duration (Days)	Physiological parameters	Sea-level performanc improvement?
			Intermittent exposure - Oxidative adaptations	
Terrados ('88)	2300	21-28	= CS/HAD	No
Terrados ('90)	2300	28	↑myoglobin (+8%†), ↑CS (+29%†),↑HAD (+22%†), ↑ASAT(+26%†)	Yes
			Intermittent exposure - Glycolytic adaptations	
Terrados ('88)	2300	21-28	↓PFK (-18%†), ↓LD (-25%†),=CK	No
Terrados ('90)	2300	28	↓LD (-16%†), ↓CK(-7%†)	Yes
Favier ('95)	3600	42	↑buffer capacity	No
			Continuous exposure - Oxidative adaptations	
Boutellier ('82)	0-8398	84	↓SDH (-45%†)	Not reported
Young, '84	4300	18	=MD	Not reported
Mizuno ('90)	2100-2700	14	↓CS/↓HAD -13%†/-10%†	Yes
Green ('92)	4300	9	= SDH / HAD	Not reported
			Continuous exposure - Glycolytic adaptations	
Young ('84)	4300	18	=phosph/HK/LD	Not reported
Ceretelli ('85)	5350	21	↓buffer capacity	Not reported
Sutton ('88)	6098	25	↓buffer capacity (-30%)	Not reported
Mizuno ('90)	2100-2700	14	↓phosph (-12%†), =LD ↑buffer capacity (+6%†)	Yes
Green ('92)	4300	9	↓PFK-14%†, ↑HK+16%†,	Not reported
Saltin ('95)	2100	14-28	=LD_{1-2}, ↓LD_{4-5}†	Not reported

Oxidative enzymes:
CS - citrate synthase
HAD - 3 hydroxy-Coacyl-dehydrogenase
ASAT - aspartate aminotransferase
SDH - succinate dehydrogenase
MD - malate dehydrogenase
↑/↓/ - increase/decrease

Glycolytic enzymes:
PFK - phosphofructokinase
$LD/LD_{1,2/4,5}$ - lactate dehydrogenase
CK - creatine kinase
Phosph - phosphorylase
HK - hexokinase
= - no change

† - Significantly different from pre-altitude value ($P < 0.05$)

2.5.3.4 Muscle Buffer Capacity

Muscle buffer capacity has been shown to decrease during continuous exposure to high altitude (5,350-6098 m) whereas increases have been reported during intermittent and chronic exposure to moderate altitudes of between 2,100 to 3,600 m (Table 2.5). This may be related to the more pronounced metabolic compensation for respiratory alkalosis experienced at higher altitudes.

In a single group design, triceps and gastrocnemius muscle buffer capacities increased by 6% ($P < 0.05$) in 10 elite cross-country skiers who spent 14 days at 2,100 to 2,700 m (Mizuno et al 1990). Whilst $\dot{V}O_{2max}$ did not change 2 days following return to sea-level, maximal O_2 deficit increased by 29% ($P < 0.05$ vs pre-altitude value) which corresponded to the equivalent of 1 L of O_2. The authors also reported a significant correlation between the increase in muscle buffer capacity and running time to exhaustion during a $\dot{V}O_{2max}$ test following return to sea-level ($r^2 = 0.83$, $P < 0.05$). Favier et al. (1995) have also reported an improvement in muscle buffering capacity and a subsequent decrease in lactacidosis following 6 weeks of intermittent hypoxic training in 30 Bolivians. The physiological mechanisms that activate increases in muscle buffering capacity and facilitate anaerobic metabolism at altitude are presently unknown. However, an increased energy yield from anaerobic glycolysis due to a decrease in $\dot{V}O_2$ kinetics at the onset of exercise (Hughson et al 1995) may be a contributory factor.

2.5.4 Substrate Utilisation

2.5.4.1 Glycogen Metabolism

Acute exposure to environmental hypoxia increases the rate of muscle glycolysis for a given submaximal work rate whereas maximal rates do not appear to change, at least up to an altitude of 4,270 m (Coudert et al 1992). Subsequent concentrations of blood lactate are increased (Figure 2.9).

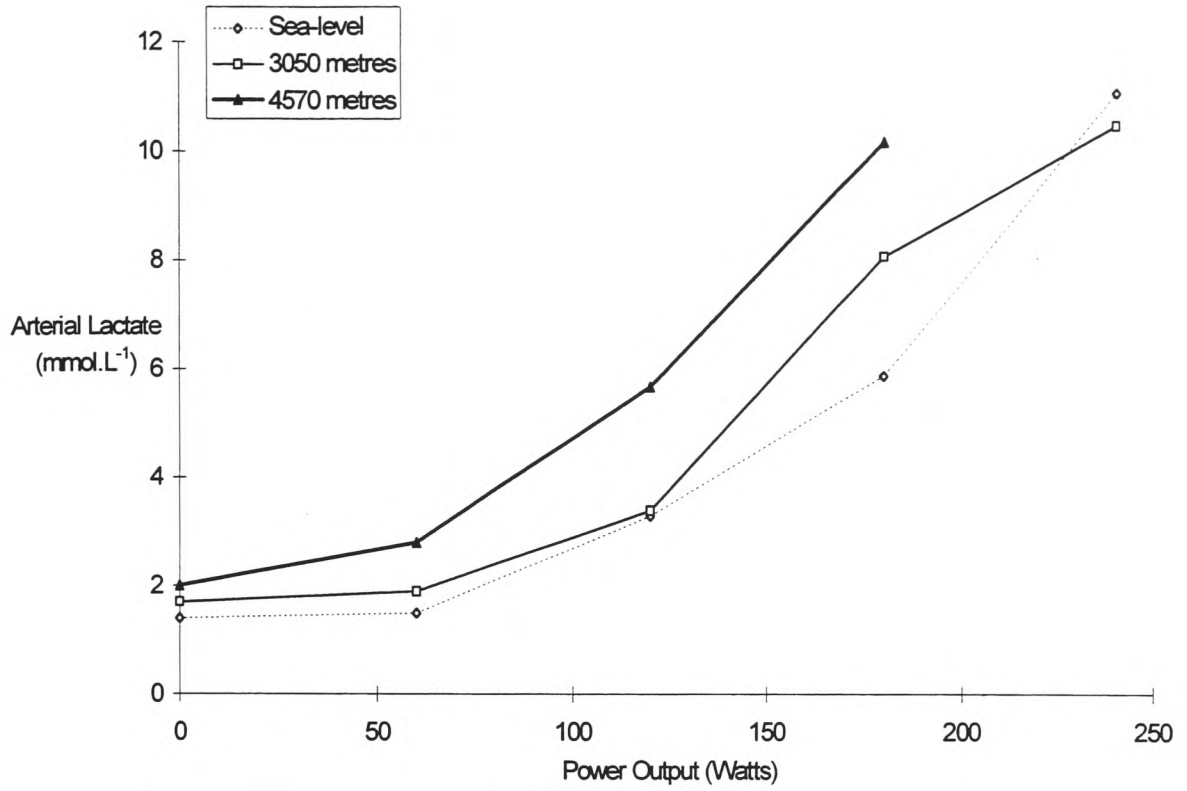


Figure 2.9 Effects of Acute Hypobaric Hypoxia (Ambient PO_2 = 90 - 159 mmHg) on Arterial Lactate Concentration (Wagner et al 1986)

Values are Means (n = 8)

The rate of glucose uptake by the exercising limb is also increased at altitude (Brooks et al 1995 and Roberts et al 1995) which may be related to an increased hexokinase activity (Green et al 1992). A series of elegant investigations by Mazzeo et al. (1989) and Young et al. (1991) have identified that the accelerated glycolytic flux is in part mediated by the increased blood adrenaline concentrations. Mazzeo et al. (1989) found a high correlation between adrenaline and lactate concentration at altitude (r = 0.95) and a follow up study by Young et al. (1991) showed a reduction in circulating lactate concentrations following β-blockade. The aetiology of β-adrenergic stimulation at 4,300 m was recently found to be related to ventilatory parameters, possibly increased chemoreceptor activity and not to measures of hypoxaemia (Asano et al 1997).

2.5.4.2 Glycogen Resynthesis

A more pronounced reduction in intramuscular glycogen stores at rest and during submaximal exercise has been observed within 4 h of ascent to 4,300 m (Green et al 1992). To this author's knowledge, only one investigation has quantified the effects of hypoxia on

muscle glycogen resynthesis following exercise. Milledge et al. (1976) studied the rate of glycogen resynthesis in type I and type II muscle fibres in 3 active mountaineers. Subjects breathed either a hypoxic gas mixture (F_IO_2-10%, ~6,000 m) or ambient air during a 5 h recovery period following 2 h of exhausting exercise at heart rates equivalent to 145-165 $b.min^{-1}$. Whilst their findings were not statistically significant due to the insufficient degrees of freedom, the authors reported a greater increase in overall muscle glycogen content following the normoxic (returned to 62% of pre-exercise control values) as opposed to the hypoxic recovery (51% of pre-exercise control values) period. Their results also suggested that hypoxia depressed glycogen resynthesis in Type I but not in type II muscle fibres which they concluded may contribute to the sensation of muscle fatigue at altitude. It is tempting to speculate that a more pronounced depression of glycogen resynthesis may predominate during chronic exposure to altitude where total food intake and carbohydrate intake may decrease by 10-50% (Brouns, 1992). Testosterone is also implicated in the conversion of inactive glycogen synthase into its active form (Adolfsson, 1971) and thus the depression of this anabolic hormone which has been noted at altitude (Sawhney et al 1985) in conjunction with inadequate food and water intake (Ratzin Jackson et al 1988) may delay the replenishment of the muscle's carbohydrate stores.

2.5.4.3 The Lactate Paradox

Altitude acclimatisation causes a reduction in submaximal and maximal blood lactate concentrations in spite of the prevailing hypoxia; an observation which was first described by H.T. Edwards during an expedition to Chile in 1935 (Edwards, 1936) and has since been termed the "lactate paradox" (Hochachka et al 1989). A number of hypotheses have been proposed to explain the reduction in glycolytic flux at altitude, several of which have been recently challenged: [1 a decrease in buffering capacity due to renal compensation of respiratory alkalosis; it was hypothesised that a reduction in intracellular pH would inactivate key enzymes of anaerobic glycolysis (Cerettelli et al 1985 and West, 1986). However, two recent investigations have demonstrated that exogenous supplementation of $NaHCO_3$ equivalent to 0.3 g/kg/body mass did not normalise maximal lactate concentrations (Kayser et al 1993a and Grassi et al 1995); [2 a reduction in muscle glycogen stores does not appear to be implicated in the lactate paradox. Butterfield et al. (1992) demonstrated that meticulous dietary control at altitude did not affect maximal blood lactate concentrations; [3 Grassi et al (1995) identified that a decrease in muscle mass at altitude was not the major determinant of the lactate paradox. He demonstrated

that maximal lactate concentrations after 1 week at 5,050 m decreased by 64% ($P < 0.05$) despite only a 2% decrease in lean body mass.

Recent data has suggested that an "upstream" inhibition of glycolysis regulated by a decreased central drive (Kayser et al 1994) and/or changes in β-adrenergic sensitivity of glycolysis (Brooks et al 1992 and Reeves et al 1992) are the major factors responsible for the lactate paradox. In contrast to sea-level measurements, Kayser et al. (1994) identified that the quadriceps muscles did not show any signs of electromyographic fatigue during exhaustive exercise following 4 wk at 5,050 m, suggestive of a central inhibition of neuromuscular activation. During this period, arterial lactate concentrations decreased by 41% ($P < 0.05$) in comparison to pre-altitude values. Administration of 100% oxygen normalised these responses, suggesting that the decrease in neuromuscular activation was mediated by hypoxia *per se*. The origin of the signals responsible for a decrease in central drive at altitude is presently unclear but it has been suggested that the respiratory system may inhibit the maximal activation of the locomotory muscles via some undetermined negative feedback system (Bigland-Ritchie et al 1988). This constitutes a useful adaptation which protects the subject against the potential damage of extreme metabolic acidosis (Grassi et al 1995).

Several well controlled studies have suggested that a decreased β-adrenergic stimulation is implicated in the lactate paradox (Reeves et al 1992). By blocking β-adrenergic receptors, Young A.J. et al. (1991) investigated the potential role of adrenaline in the physiological mechanisms that regulate lactate flux at altitude. They demonstrated that in contrast to a control group who received a placebo, subjects who received propranalol (240 mg/day) did not exhibit a decrease in blood lactate at 80% $\dot{V}O_{2max}$ between days 3 to 15 at 4,300 m. In a separate study using isotopic tracers, ([6,6-2D] glucose and [3-13C] lactate) Brooks et al. (1991) demonstrated that in comparison with acute hypoxia, the rate of blood lactate appearance decreased during exercise at 65% $\dot{V}O_{2max}$ following 3 weeks of acclimatisation to 4,300 m. The decrease in blood lactate concentration was correlated with a decrease in adrenaline concentration ($r = 0.88$).

Saltin (1996) has suggested an alternative mechanism. He has proposed that the lactate paradox *itself* causes a decrease in sympathetic activity and not vice versa. The functional

significance of this is not understood and warrants further investigation. However, if one considers that a decrease in muscle pH activates the sympathetic nervous system (Victor et al 1988), a regulating factor during a prolonged stay at altitude may involve the inhibition of muscle glycogenolysis. The subsequent decrease in sympathetic activation would be of functional significance as it would reduce the chronotropic effect of the heart and improve $\dot{V}_A/\dot{Q}_C$ at altitude (Section 2.5.2.3).

Whatever the mechanism(s) involved in the lactate paradox, ATP homeostasis is maintained at altitude despite the reduction in glycolytic flux (Green et al 1992); free ADP was shown to decrease and the ATP-to-free ADP ratio increased ($P < 0.05$) following 3 wks of acclimatisation to 4,300 m which would suggest a tighter coupling between oxidative phosphorylation and glycolytic flux. Decreased blood lactate concentrations have been shown to predominate even after 8 d at sea-level (Beidleman et al 1996).

2.5.4.4 Protein Metabolism

Large decreases in muscle mass have been noted at altitude (Hoppeler et al 1990). Whilst up to 30% of these losses can be attributed to water loss, detraining or malnutrition (Kayser, 1994) hypoxia *per se* has been demonstrated to increase protein oxidation and depress protein synthesis (Rennie et al 1983). Whilst modulation of protein metabolism may be mediated via endocrine and paracrine hormones, there is evidence to suggest that changes are due to the direct effects of hypoxia *per se* (Preedy et al 1985). The physiological mechanisms responsible for these changes are not currently understood (Narici and Kayser, 1995).

An increased oxidation of branched chain amino acids (BCAA) has been noted at altitude (Bigard et al 1993, 1996). These changes in the plasma profile of amino acids could increase concentrations of 5 hydroxytryptamine (5-HT) in the brain (Newsholme et al 1987) which is implicated in the genesis of central fatigue. Wagenmakers (1992) has also suggested that sympathetically-mediated glycogen depletion at altitude would ultimately result in depressed glutamine metabolism due to a shortage of 2-oxoglutarate. This has important implications since glutamine is required by key cells of the immune system, in particular lymphocytes and macrophages (Ardawi et al 1985). A recent investigation by Bigard et al. (1996) has demonstrated that daily supplementation with BCAA can attenuate

muscle atrophy by maintaining a positive nitrogen balance and thus maintain maximal cycling power output during an altitude sojourn to 2,500 - 4,100 m.

2.5.4.5 Lipid-Lipoprotein Metabolism

The effects of altitude training on blood lipid metabolism is controversial. Young A.J. et al. (1982) and Young P.M. et al. (1987, 1992) have demonstrated decreases in blood lactate and ammonia concentration and an enhanced oxidation of free fatty acids (FFA) during cycling exercise at 75% VO_{2max} following 3 weeks of acclimatisation to 4,300 m. An increased serum FFA concentration and decreased RER during steady state exercise has also been noted for up to 8 d following return to sea-level (Beidleman et al 1996 and Bigard et al 1996). These changes may be related to the lipolytic effects of catecholamines and thyroxine which have been shown to increase at altitude (Ferezou et al 1988). Whitten et al. (1969) identified a significant negative correlation between serum FFA and caloric intake (r = -0.69, $P < 0.001$) during a 9 day mountaineering sojourn to 4,299 m. It would appear that caloric restriction at altitude may also contribute to the enhanced lipolysis. It has been suggested that an enhanced fat oxidation at altitude is a physiological measure which serves to decrease the utilisation of muscle glycogen (Ratzin Jackson et al 1988). However, Brouns (1992) believes that it is a consequence of a negative energy balance mediated by reduced total energy intake and hormonal shifts towards a catabolic state.

In contrast, isotope infusion studies have demonstrated that acute and chronic exposure to hypoxia (4,300 metres) increased blood glucose uptake but decreased FFA uptake during submaximal exercise (Roberts et al 1995 and Brooks et al 1995). An increased preference for and oxidation of dietary carbohydrate is a useful adaptation at altitude that increases SaO_2 by increasing PAO_2 at any given $PACO_2$. (Ward et al 1995).

High altitude natives have a lower incidence of coronary artery disease and myocardial infarction (Shivastava and Malhotra, 1974; Mortimer et al 1977 and Sun, 1985). Epidemiological studies conducted in S.America have demonstrated that ECG evidence of myocardial ischaemia is significantly lower than at sea-level (Ramos et al 1967). De Mendoza et al. (1979) compared plasma cholesterol concentrations in socioeconomically matched high (3,500 m) and low (1,000 m) altitude Venezuelian subjects. They showed that the low altitude residents had higher total cholesterol concentrations, predominantly LDL-C. Although genetic and behavioural factors are implicated, lower serum cholesterol

concentrations and an inverse relationship between coronary mortality and altitude of residence (Voors et al 1979) may suggest a role for hypobaric hypoxia as a potential modulator of blood lipid metabolism. However, few investigations have quantified changes in the lowlander's blood lipid-lipoprotein profile during an acute sojourn to altitude.

Table 2.6 summarises the effects of altitude training on the resting blood lipid-lipoprotein profile of the native lowlander. Mountaineering studies have generally shown improvements in the blood lipid profile (Balke et al 1965; Nestel et al 1979 and Ferezou et al 1988). Ferezou et al. (1988) investigated changes in plasma lipids and lipoprotein cholesterol in 8 subjects who spent 3 weeks at 4,800 m. In comparison to sea-level values, they observed a significant decrease in total cholesterol (TC), phospholipids, triglycerides and low density lipoprotein (LDL-C) whereas high density lipoprotein (HDL-C) increased significantly ($P < 0.05$) by the end of the altitude sojourn. The authors attributed these metabolic changes to concomittant increases in resting plasma noradrenaline and thyroxine concentrations. Catecholamines stimulate lipolysis (Hietanen, 1982) and an increased thyroxine concentration typical of patients with hyperthyroidism has been shown to increase the receptor-mediated uptake of LDL-C (Heimberg et al 1985). However, their conclusions that hypoxia *per se* was responsible for these cardioprotective adaptations can remain only speculative since they failed to control for a variety of confounding factors that include cold, malnutrition and increases in physical activity.

In contrast, a controlled investigation as part of Operation Everest II by Young et al. (1989) demonstrated significant increases in plasma triglycerides and a decrease in HDL-C during a 40 day exposure to hypobaric hypoxia (~4,300 m to 7,615 m) . These changes persisted following return to sea-level conditions. Young et al. (1989) concluded that an increase in plasma insulin concentration at altitude stimulated triglyceride synthesis by the liver and decreased catabolism of triglyceride rich lipoproteins possibly by depressing the activity of lipoprotein lipase. This is clearly an area of research which requires further investigation.

Table 2.6 Blood Lipid-Lipoprotein Metabolism At Altitude

Author	Altitude	Duration	Physiological changes:	
(Year)	(Metres)	(Days)	Lipids	Lipoprotein
Balke (1965)	2300	10	↓ TC (-10%)	ND
Whitten (1969)	4299	9	=TC/ ↑Tg* (+34%)	ND
Bason (1969)	3800	3	↑TC‡ (+21%)	ND
Nestel (1979)	4000-7130	56	=	↑ ApoAI* (+92%)
Ferezou (1988)	4800	3	↓ TC* (-27%)	↓ LDL* (-38%)
Ferezou (1988)	4800	20	↓ TC* (-22%)	↓ LDL* (-34%)
			↓ Tg* (-41%)	↑ HDL* (+6%)
Young (1989)	4300	7	=	=
	5450	7	↑ Tg‡ (+64%)	=
	6200	11	↑ Tg‡ (+66%)	=
	7615	15	↑ Tg† (+45%) ↓TC‡	↓ HDL‡ (-30%)
			(-25%)	↑ VLDL‡ (+82%)

TC - total cholesterol
Tg - triglycerides
HDL - high density lipoprotein
VLDL - very low density lipioprotein
LDL - low density lipoprotein
ND - No data
= - no changes relative to normoxic value
↓ - decrease relative to normoxic value
↑ - increase relative to normoxic value
† - Significantly different to pre-altitude value ($P < 0.05$)
‡ - Significantly different to pre-altitude value ($P < 0.01$)
* - Significantly different to pre-altitude value ($P < 0.001$)

2.6 PHYSICAL CAPACITY AT ALTITUDE

2.6.1 Maximal Performance

A common observation at altitude is the reduction in maximal aerobic capacity ($\dot{V}O_{2max}$). It would appear that maximal performance decreases when SaO_2 decreases below a threshold value of 87% (Kouskolou et al 1994). The decrease in $\dot{V}O_{2max}$ at altitude is depicted in Figure 2.10. These data would suggest that $\dot{V}O_{2max}$ is reduced on average by 8-9% for every 1000 m ascent above sea-level. However, since the first observation by Saltin et al (1968), several investigators have since identified a more pronounced decrement in $\dot{V}O_{2max}$ at altitude in the elite endurance athlete (Lawler et al 1988; Shephard et al 1988 and Koistinen et al 1995). In fact, a recent study by Gore et al. (1996) has demonstrated that $\dot{V}O_{2max}$ was 7% lower ($P < 0.05$) even at 580 m above sea-level in elite endurance athletes with a sea-level $\dot{V}O_{2max}$ of 77 ± 1.ml.kg.$^{-1}$ min^{-1}. Several mechanisms have been proposed to explain these findings which include hypoventilation, venoarterial shunting, ventilation-perfusion inequality and an alveolar capillary-diffusion limitation (Rowell et al 1964 and Dempsey, 1986).

Physiological adaptations invoked during altitude acclimatisation, whilst improving the physiological status at rest, are apparently ineffective at improving $\dot{V}O_{2max}$ (Figure 2.10). Whilst a decrease in total blood volume and maximal heart rate may be implicated in this response (Saltin, 1996), experimental evidence would suggest that an increase in sympathetically-mediated vasoconstriction is the dominant factor that regulates oxygen transport at altitude (Boutellier et al 1982 and Wolfel et al 1991).

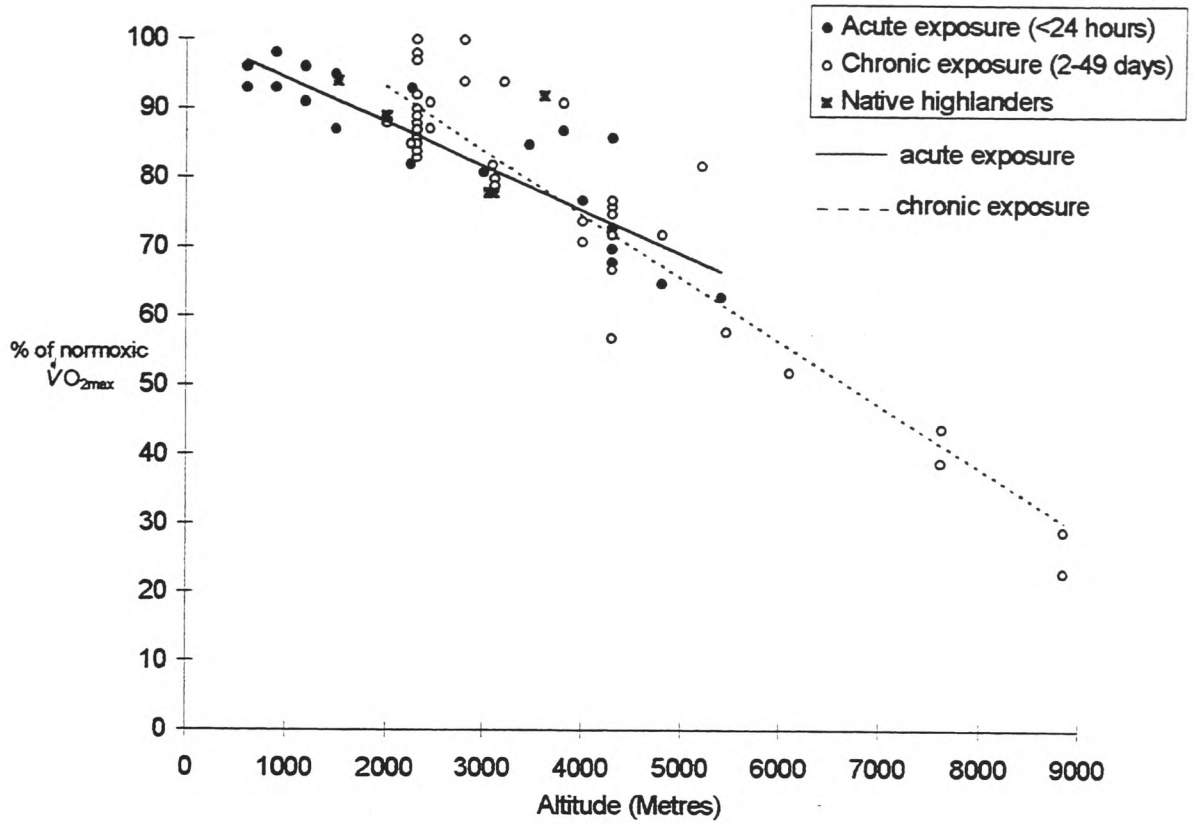


Figure 2.10 Effects of Acute and Chronic Hypoxia on Maximal Oxygen Uptake ($\dot{V}O_{2max}$) in Native Lowlanders and Highlanders

Data based on: Adams et al. (1975), Asahina et al. (1966), Balke et al. (1965), Bender et al. (1989), Benoit et al. (1992), Boutellier et al. (1990), Daniels et al. (1970), Desplanches et al. (1993), Dill et al. (1971), Faulkner et al. (1967, 1968), Favier et al. (1995), Fulco et al. (1994), Gore et al. (1996), Grover et al. (1967), Hansen et al. (1967), Klausen et al. (1966), Koistinen et al. (1995), Reeves et al. (1967), Roskamm et al. (1969), Saltin et al. (1968), Saltin et al. (1995), Sucec et al. (1996), Sutton et al. (1988), Svedenhag et al. (1991), Terrados et al. (1992), Tucker et al. (1984), West (1983), Wolfel et al. (1991), Young A.J. et al. (1996), Young P.M. et al. (1987, 1992), Vallier et al. (1996),

Wolfel et al. (1991) examined the regulation of systemic oxygen transport during acute (< 4 h) and chronic (21 d) exposure to 4,300 m. They identified that whilst CaO_2 increased by 20% ($P < 0.05$) after 21 d at 4,300 m, leg blood flow determined using a thermodilution technique (Bender et al 1988 and Sullivan et al, 1987) during steady state exercise decreased by 18% ($P < 0.05$) due to an increase in central and peripheral vascular resistance. The decrease in blood flow was independent of blood volume and occurred in response to an 18% ($P < 0.05$) increase in noradrenaline concentration. The overall result was an unchanged total body and leg $\dot{V}O_2$ (Figure 2.11).

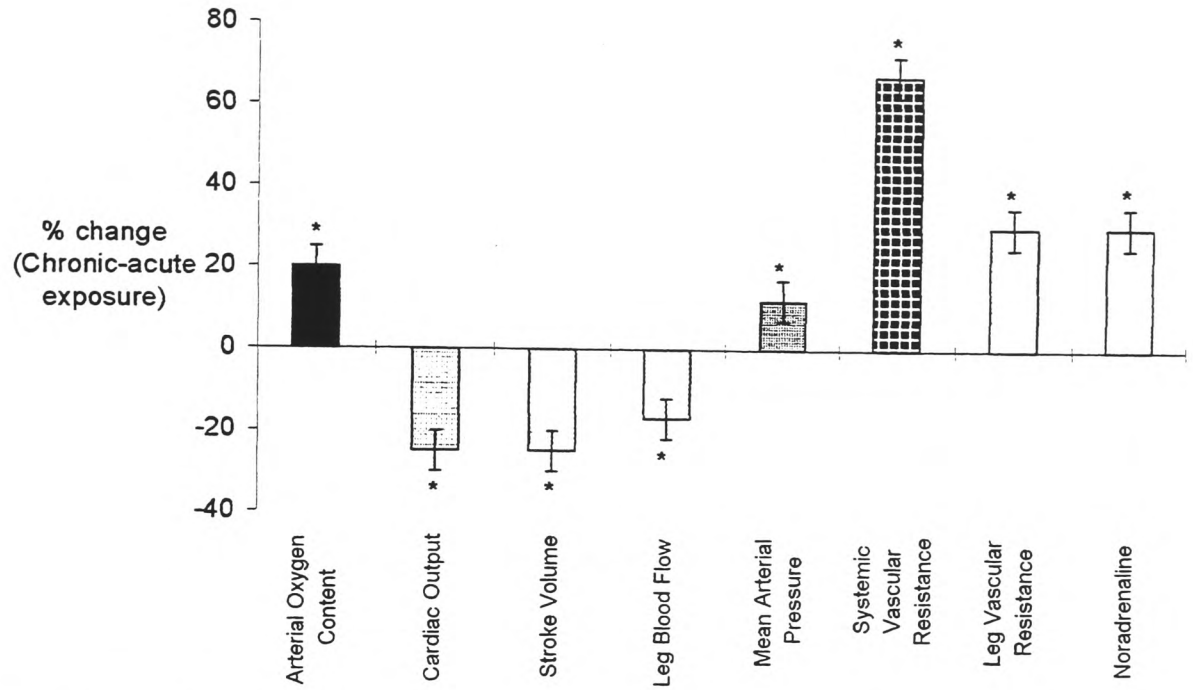


Figure 2.11 Major Cardiovascular Changes During Altitude Acclimatisation (Wolfel et al, 1991)

Values are Mean ± SD based on 7 subjects
* - significantly different from pre-altitude value ($P<0.05$)

The significance of active vasoconstriction at altitude is at present poorly understood. Whilst it may improve ventilation-perfusion characteristics in the lung, it may also prove detrimental as it has been implicated in the genesis of pulmonary oedema and cerebral venous thrombosis (Ward et al 1995).

2.6.2 Submaximal Performance

Whilst $\dot{V}O_{2max}$ is an important determinant of an individual's capacity to perform endurance exercise, other metabolic and cardiorespiratory measurements obtained during *submaximal* exercise as markers of *"economy"* have been demonstrated to correlate more highly with performance (Noakes, 1988).

It would appear from the data presented in Table 2.7 that blood lactate, plasma ammonia and heart rate at a given submaximal power output decrease and as a consequence, exercise time to exhaustion increases during prolonged exposure to altitude. These adaptations have been linked to alterations in substrate availability to predominantly fat oxidation, a decrease in β-sympathetic activity or a decrease in central drive (Section 2.5.4.3). Contrary to recent

comments by Saltin (1996), whether hypoxia per se is implicated is at present unresolved. With the exception of the study by Svedenhag et al. (1991), the remaining studies presented in Table 2.7 have failed to incorporate a normoxically trained control group in the experimental design. It would therefore seem equally possible that the performance improvements reflect an habituation and/or a training effect.

Table 2.7 Physiological Correlates of Submaximal Performance During Altitude Acclimatisation in Native Lowlanders

Variable measured	% of acute ascent value (*)	Exercise mode/ Intensity	Altitude (Metres)	Author (Year)
Exercise time to exhaustion	107 (10)	Cycle at 20 METS	2800	Balke '65
	106(10)116 (50)	Incremental cycle test to exhaustion	4000	Buskirk '67
	116 (14)	Cycle at 160W	4300	Fulco '94
Heart rate	100(3) 93‡ (25)	Cycle at 240W	2240	Saltin '68
	92‡ (28)	Cycle at 135W	3600-6500	Stoneham '93
	96 (14)	Cycle at 160W	4300	Fulco '94
	82‡ (9)	Run 70%$\dot{V}O_{2max}$	4300	Young '96
Blood lactate	104 (3) 65† (25)	Cycle at 240W	2240	Saltin '68
	71† (18)	Cycle at 123W	4300	Bender '89
	70† (13)	Run at 4.44 $m.s^{-1}$ inclination 2.8%	2000	Svedenhag '91
	50‡ (9)	Run 70% $\dot{V}O_{2max}$	4300	Young '96

† - significantly different from acute ascent value ($P < 0.05$)
‡ - significantly different from acute ascent value ($P < 0.01$)
(*) - figures in brackets represent days spent at altitude

2.6.3 Supramaximal (Anaerobic) Performance

Relatively few investigations have studied the effects of altitude acclimatisation on supramaximal (>$\dot{V}O_{2max}$) or anaerobic performance. Quantification of this physiological parameter is difficult as no reliable method is currently available that accurately measures anaerobic metabolism (Lakomy, 1994).

However, Saltin (1996) has suggested that an increase in muscle buffer capacity (Mizuno et al 1990; Favier et al 1995; Saltin et al 1995 and Nummela et al 1996) should theoretically increase anaerobic capacity and thus account for the improvements in time trial performance (mean velocity attained during supramaximal exercise) that have been observed in previous studies (Table 2.8). However, definitive conclusions cannot be made at present due to the lack of normoxically trained control groups and insufficient degrees of freedom (Pugh, 1965; Faulkner et al 1967, 1968 and Daniels et al 1970).

Table 2.8 Supramaximal Exercise Performance During Altitude Acclimatisation in Native Lowlanders

Sample Size (n)	% of acute ascent value*	Exercise mode/ Intensity	Altitude (Metres)	Author (Year)
3	106(14)107(21)	800 metre run	2300	Faulkner '67
4	104 (21)	1 mile run		
3	104 (14)	2 mile run		
5	100(3) 100 (14)	100 yard swim	2300	Faulkner '67
8	101(3) 101 (14)	200 yard swim		
3	103(3) 102 (14)	500 yard swim		
6	107 (7)106 (32)	1 mile run	2300	Faulkner '68
4	108(14)106(32)	2 mile run		
4	106 (7)104 (32)	3 mile run		
6	103(25)104(35)	1 mile run	2300	Daniels '70
6	101(25)102(35)	3 mile run		
6	101(12)102(30)	2 mile run	2150	Pugh '65

* - figures in brackets represent days spent at altitude

Laboratory methods that have been used to determine anaerobic performance at altitude have focused on [1 evaluation of anaerobic capacity by determining maximal oxygen deficit/debt and maximal concentrations of blood lactate, and [2 measurement of external mechanical power output. Maximal oxygen deficit/debt have been demonstrated to underestimate anaerobic capacity and its measurement at altitude is considered to be of limited use (Saltin, 1996). Indirect evaluation of anaerobic capacity from maximal lactate

concentrations are confounded by the lactate paradox and an apparent disassociation has been observed between muscle and blood lactate at an altitude of between 4,300 to 4,880 m; a possible consequence of a decreased release or an increased rate of lactate oxidation (Bender et al 1989 and McLellan et al 1990).

Probably the most valid determinant of anaerobic power at altitude involves quantification of external mechanical power output. Peak and mean power output (P_{max}, P_{mean} respectively) have been measured during short intense exercise lasting less than 10 s (alactic component) or during maximal exercise lasting for 30 s or more (lactic component) during a Wingate test (Coudert et al 1992). A summary of the major research findings are illustrated in Table 2.9.

Table 2.9 External Mechanical Power Output at Altitude

Author (Year)	Altitude (Metres)	Duration (Days)	Exercise Mode	Physiological Changes
Cunningham '70	2200	4-22	Wingate test	=
Blonc '94	3600	1	Wingate test	$=P_{max}/=P_{mean}$
DiPrampero '82	4340	21	Cycle sprint	=
Kavanagh '86	4400	1	Wingate test	$\downarrow P_{mean}$†
Young '80	4570	2	Isokinetics	=
McLellan '90	4800	1	Wingate test	=
Ferretti '90	>5000	56	Sargeant jump	$\downarrow P_{max}$†/$\downarrow_{mean}$†*
Kayser '93c	5050	28	Sargeant jump	=
Kayser '94	5050	28	Forearm flexion	=
Cerettelli '85	5200	21-56	Sargeant jump	$\downarrow P_{max}$†*
Richalet '92	6542	21	Wingate test	$=P_{max}/\downarrow P_{mean}$†

* - no difference when expressed relative to lean body mass
↓ - decrease
† - significantly different from pre-altitude/normoxic value ($P < 0.05$)
= - no change
P_{max} - maximal power output
P_{mean}- mean power output

Assuming that the aerobic contribution during a 30 s Wingate test accounts for 13 to 28% of the total energy yield (Bar-Or, 1987 and Vanderwalle et al 1987), the decrease in P_{mean} noted at altitude (Kavanagh et al 1986 and Bedu et al 1991) is probably due to a decreased contribution from aerobic metabolism (Bedu et al 1991). Anaerobic alactic performance has also been demonstrated to decrease at altitude but these changes would appear to be related to a concomitant decrease in skeletal muscle tissue mass (Cerettelli et al 1985 and Ferretti et al 1990). Expression of power output per unit of cross-sectional area of muscle has identified that muscle per se does not lose its ability to generate power at altitude (Ferretti et al 1990) and hence neuromuscular performance remains intact. This has been supported by Kayser et al. (1993) who demonstrated that alpha-motorneuron excitability, nerve and muscle fibre conduction velocity and synaptic and muscle end plate transmission were all maintained during acclimatisation to 5,050 m.

The evidence to date would suggest that anaerobic performance is unaltered up to an altitude of 5,000 m. However, long sojourns to higher altitudes results in a decrease in muscle mass (Kayser, 1994) and a subsequent reduction in muscle fibre diameter due to a loss of myofibrillar protein content (Hoppeler et al 1990) has been shown to decrease anaerobic performance.

2.7 THE EFFECTS OF ALTITUDE TRAINING ON SEA-LEVEL ENDURANCE PERFORMANCE IN NATIVE LOWLANDERS

Ninety one investigations have quantified the effects of altitude training on sea-level endurance performance, dating back from the present day to 1956. These studies have incorporated 772 altitude trained experimental and 209 sea-level trained control subjects. As illustrated in Figure 2.12, only 27 (30%) of the 91 hypoxic training studies reviewed have incorporated a normoxically trained control group. This makes it impossible to discriminate whether the physiological changes that occur following a bout of altitude training can be attributed to an improvement in physical conditioning or to the additive effects of hypoxia itself.

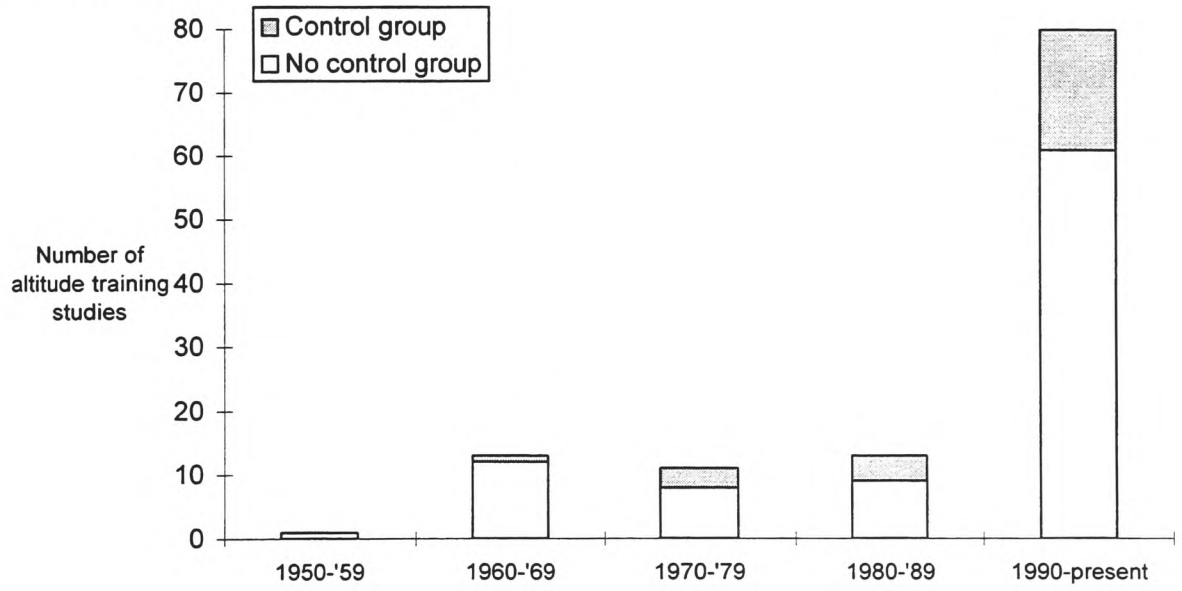


Figure 2.12 Frequency of Altitude Training Studies with a Normoxically Trained Control Group

The effects of altitude training on sea-level endurance performance is summarised in Tables 2.10 and 2.11. These data were classified according to the characteristics of the hypoxic stimulus which included: duration, magnitude (calculated ambient PO_2) and timing of physiological testing following the descent to sea-level.

2.7.1 Potentiating Effects

2.7.1.1 Aerobic Performance

To the author's knowledge, the altitude training studies conducted by Asano et al. (1986), Terrados et al. (1988, 1990) and Levine et al. (1996) would appear to be the only investigations employing a control group that have reported statistically significant improvements in aerobic performance following return to normoxia (Table 2.10).

Table 2.10 Potentiating Effects of Hypoxic Training on Sea-Level Endurance Performance

Author /Year	Altitude (Metres)	Exposure Time (Days)	Submaximal Improvement	$\Delta\dot{V}O_{2max}$ Post-Pre %	Control Group
Klausen '66	3800	35	ND	+14†	*No*
Faulkner '67	2300	23	Yes†	+8†/+10†	*No*
Loeppky '70	3049-4268	23	Yes†	NS	*No*
Banister '78	4020	21/28	Yes†	+8‡/+26‡	*No*
Mizuno '90	2100-2700	14	Yes†	NS	*No*
Ingjer '92	1900	21	Yes†	NS/NS	*No*
Stray. G '95	2500	28	ND	+6†	*No*
Levine '91	1300-2500	28	Yes†	+4†	*No*
Stray.G '97	1250-3000	28	Yes†	+2.5*	*No*
Asano '86	4000	70	Yes†	NS	*Yes*
Terrados '88	2300	21-28	Yes†	NS	*Yes*
Terrados '90	2300	28	Yes†	ND	*Yes*
Levine '96	1250-2500	28	Yes†	+4†	*Yes*

ND - No data
* - level of significance not reported
† - significantly different from pre-altitude value ($P < 0.05$)
‡ - significantly different from pre-altitude value ($P < 0.01$)
NS - Not significantly different from pre-altitude value ($P > 0.05$)

Asano et al. (1986) studied ten elite middle to long distance male runners, who trained for a 10 week period at the same relative exercise intensity at either sea-level or at a simulated altitude of 4,000 metres. Following training, there were no improvements in $\dot{V}O_{2max}$ at sea-

level, yet 10 km personal best running times improved by approximately 6%, ($P < 0.05$). Using a one legged training model, Terrados et al. (1988, 1990) attributed the potentiating effects of intermittent hypobaric training to increases in citrate synthase activity and myoglobin content; four weeks of intermittent exposure to an altitude of 2,300 m resulted in a 21% greater increase ($P < 0.05$) in endurance time to exhaustion at 80% $\dot{V}O_{2max}$ in the hypoxic as opposed to the normoxically-trained leg. Levine et al. (1990, 1991, 1996,1997) have suggested that the synergistic effects of hypoxia and training have been precluded by decreases in absolute training intensity at altitude. In their most recent study, (Levine et al 1996,1997) 39 competitive runners were randomly assigned to four weeks of [1] living high (2500 m), training low (1,250 m) [2] living high (2,500 m), training high (2500 m) or [3] living low (150 m), training low (150 m). The authors demonstrated that although $\dot{V}O_{2max}$ values significantly improved by 4% in the two altitude trained groups, 5km race performance times, the running velocity that corresponded to $\dot{V}O_{2max}$ and the ventilatory threshold at sea-level were significantly improved only in the group that lived high and trained low. An unusual finding was that 5 km performance time was 31 seconds slower in the sea-level control group which may suggest that the training stimulus was not absolutely controlled during the experimental period. Nevertheless, it was concluded that the potentiating effects of altitude training were due to a high altitude acclimatisation effect (improved haematology) and a low altitude training effect (increased training intensity). Thus, the authors advocated the practice of "living high and training low" as the optimal approach to altitude training. Whether a similar strategy is beneficial to "elite" endurance athletes remains speculative and further research is warranted to endorse this approach to altitude training. It has also been suggested that medical problems may arise when athletes fluctuate between sea-level and altitude (Wolski et al 1996).

2.7.1.2 Anaerobic Performance

Whilst previous investigations have dealt primarily with aerobic responses to altitude training, there is some evidence to suggest that anaerobic performance is improved on return to sea-level (Mizuno et al 1990; Martino et al 1994 and Nummela et al 1996). Mizuno et al. (1990) demonstrated that exercise time to exhaustion following altitude training improved by 17% ($P < 0.05$) when compared to pre-altitude values which they attributed to a 6% increase ($P < 0.05$) in muscle buffer capacity. However, the validity of these findings is questionable due to the lack of a normoxically trained control group

incorporated in the experimental design. A well controlled investigation by Martino et al. (1994) which incorporated a performance matched control group based at sea-level investigated the effects of 3 weeks of altitude training at 2,800 m on anaerobic measures of swimming performance. Sea-level sprint performance time over 100 m was 2.4 s quicker in the altitude trained group when compared with the control group ($P < 0.05$). The largest improvements in the altitude trained group were noted in an upper body Wingate test. Peak power output increased by 27.9W more than control group ($P < 0.05$). In a recent investigation, Nummela et al. (1996) demonstrated that 10 days of living high (~2,200 m) and training low (sea-level) resulted in greater improvements in 400 m running time ($P < 0.05$) and running velocity at a fixed concentration of blood lactate ($P < 0.05$) when compared with equivalent sea-level training. Resting blood pH, standard base excess and HCO^-_3 concentrations increased in 5 out of 6 subjects but these changes were not significant possibly due to the small sample size.

2.7.2 No Effects

However, the vast majority of altitude training studies have not identified performance improvements at sea-level (Table 2.11). Whilst a decrease in absolute training intensity may be implicated in the general lack of potentiating effects (Stine et al 1992), a decrease in muscle perfusion also appears to play a contributory role (Section 2.9.1). Boutellier et al. (1982) investigated the effects of a 3 month sojourn to 8,398 m on submaximal and maximal performance following return to sea-level in 8 subjects. They demonstrated that muscle blood flow determined using ^{133}Xe decreased by 26% ($P < 0.05$) and 39% ($P < 0.001$) during a step test at 75W and 125W respectively. They also identified that $\dot{V}O_2$ on-response kinetics (time required to achieve 50% of the steady state $\dot{V}O_2$) increased post altitude by 10.4 s ($P < 0.01$), which they attributed to a 45% decrease in succinate dehydrogenase activity ($P < 0.05$). However, it is clear from their findings that the decrease in muscle perfusion was not the sole result of a decrease in $\dot{Q}$; muscle blood flow was decreased at 75W whereas no changes were reported for $\dot{Q}$. The precise mechanisms reponsible for the decrease in muscle perfusion at sea-level are not known. However, an increase in vascular resistance due to an augmented blood viscosity may be a contributory factor. It is also possible that either regional or systemic vasoconstriction may have reduced blood flow to skeletal muscle in response to an increased PaO_2. Animal and human studies have shown that an elevated PaO_2 following the administration of hyperoxic gas mixtures

decreases blood flow such that total oxygen delivery to skeletal muscle remains unaltered (Welch et al 1977 and Hogan et al 1986).

Favier et al. (1995) hypothesised that the negative findings reported in the literature could, in part, be attributed to the fact that subjects were not fully acclimatised to hypobaric hypoxia. They employed three groups of sedentary high-altitude residents who trained for 30 minutes a day against a constant load on a bicycle ergometer, during a 6 week period. Group 1 trained at a PO_2 which was equivalent to an interpolated altitude of 3345 m at 70% of $\dot{V}O_{2max}$ determined in hypoxia. The remaining two groups trained under normoxic conditions at either the same relative workload, (70% of the normoxic $\dot{V}O_{2max}$) or the same absolute workload, (70% of the hypoxic $\dot{V}O_{2max}$) as the hypoxically trained group. An incremental test to exhaustion was performed by all groups in normoxia and hypoxia immediately pre and post training in an attempt to ascertain the physiological responses to sub-maximal and maximal exercise. The authors demonstrated that $\dot{V}O_{2max}$ values improved similarly in all groups. However, they hypothesised that a lower reduction in base excess and bicarbonate stores observed in the hypoxically trained group only, could potentially benefit anaerobic metabolism and, although time to exhaustion was not quantified, facilitate exercise performance.

Table 2.11 No Effects of Hypoxic Training on Sea-Level Endurance Performance

Author /Year	Altitude (Metres)	Exposure Time (Days)	Submaximal Improvement	$\Delta\dot{V}O_{2max}$ Post-Pre %	Control Group
Balke '65	2800	10	No	+7*	*No*
Asahina '66	2240	20	ND	+6/+9*	*No*
Faulkner '67	2300	14	No	NS	*No*
Reeves '67	3110	21	ND	-5*	*No*
Buskirk '67	4000	48-63	No	NS	*No*
Faulkner '68	2300	42	No	NS	*No*
Daniels '70	2300	70	ND	NS	*No*
Dill '71	3090	17	ND	NS	*No*
Klausen '91	1695-2700	7	ND	NS	*No*
Vallier '96	4000	21	ND	NS	*No*
Hansen '67	4300	28	ND	NS	*Yes*
Roskamm'69	2250	28	ND	+17.5*	*Yes*
Roskamm'69	3450	28	ND	+10.0*	*Yes*
Davies '74	4020	15	ND	NS	*Yes*
Adams '75	2300	21	No	NS	*Yes*
Rahkila '82	2600	11	NS	NS	*Yes*
Levine '90	2500	28	No	NS	*Yes*
Friedman '90	2500	35	ND	NS	*Yes*
Svedenhag '91	2000	14	ND	NS/NS	*Yes*
Hahn '92	3100	19	No	NS	*Yes*
Desplanches'93	4100-5700	21	ND	NS	*Yes*
Favier '95	3345	42	ND	NS	*Yes*
Rusko '96	1600-1800	18-28	ND	NS	*Yes*
Bailey '96	1640	28	No	NS	*Yes*
Telford '96	1700-2000	28	No	NS	*Yes*

ND - No data
* - level of significance not reported
NS - Not significantly different from pre-altitude value ($P > 0.05$)

2.8 MODULATING FACTORS OF ALTITUDE TRAINING; PHYSIOLOGICAL IMPLICATIONS FOR ENDURANCE PERFORMANCE FOLLOWING RETURN TO SEA-LEVEL

It is becoming clearer that a number of methodological deficiencies may preclude the potential synergistic effects of hypoxia and physical exercise; the physiological implications of which will be discussed in the following section.

2.8.1 Intensity and Duration of the Hypoxic Stimulus and Associated Haematological Adaptation

There is still much controversy regarding the optimal altitude and duration required for athletes to train in an attempt to optimise endurance performance following return to sea-level. Much attention has focused on the erythropoetic response to hypoxia and subsequent haematological adaptation. Considering the inverse relationship between PO_2 and resting Hb concentration (Winslow et al 1987) it would seem logical that the higher the athlete can train the better. However, other factors which inhibit exercise performance are exacerbated with a reduction in PO_2. Acute mountain sickness presents at altitudes above 2000 to 3000 m (Milledge, 1994) with the possibility of the elite athlete suffering physiological symptoms which include headache, nausea, vomiting, malaise and insomnia at even lower altitudes (Shephard, 1992). Prolonged exposure to altitudes above 4,500 m has been shown to result in a reduction in muscle mass, the underlying physiological mechanisms for which have been recently reviewed by Kayser et al. (1994) and discussed in section 2.8.4. Finally, the effects of training at a lower PO_2 may result in a reduction in training load, so that detraining may override the potential benefits of altitude acclimatisation (Saltin, 1967).

2.8.2 Hypoxia and Detraining

Saltin (1967) originally suggested that altitude exposure would result in de-training. Daniels and Oldridge (1970) demonstrated the importance of maintaining training intensity at altitude and subsequent effects on sea-level performance. They suggested that intermittent exposures to altitudes of between 2300 to 3300 m and sea-level optimised the balance between hypoxic acclimatisation and training intensity. Despite the experimental limitations of a single group design, 2 world records and 12 personal best times were recorded by athletes on return to sea-level which presented a reasonable endorsement for

such an approach. However, it is equally possible to have expected similar improvements in a control group training at sea-level.

Stine et al. (1992) and Harper et al. (1992) have quantified the detraining response at altitude. They identified that athletes trained at faster running velocities and greater percentages of $\dot{V}O_{2max}$ at a lower altitude of 1,200 m when compared to 2,800 m. The same group have controlled training intensity at altitude in a sequence of elegantly designed hypobaric chamber studies (Levine et al 1990, 1991, 1996). They demonstrated that altitude training potentiates endurance performance so long as absolute training intensity can be maintained. This has popularised the use of "altitude houses" recently developed in Finland which are portable hypobaric chambers used by elite athletes who alternate living and sleeping at simulated altitude with normobaric training (Nummela et al 1996). However, the effectiveness of this procedure should at present be considered equivocal and further scientific investigation is warranted to endorse this approach to altitude training.

2.8.3 The Concept of a Critical PO_2 and Haematological Adaptation

Few athletes can afford the costs inherent in a "live high, train low" approach to altitude training. Therefore, is it possible that a "threshold" altitude exists which optimises the benefits of haematological acclimatisation and minimises the negative effects of de-training? Weil et al. (1968) has presented the most comprehensive evidence which indicates the existence of such a threshold, albeit in sedentary, highland natives (personal communication, Dr B.Levine, University of Texas Southwestern Medical Center, Texas, USA). They identified a bi-phasic relationship between the arterial partial pressure of oxygen (PaO_2) and red blood cell mass and demonstrated a clear inflection point at a "critical" PaO_2 of 67 mmHg, equivalent to an interpolated SaO_2 of 92%. This point corresponds to the steeper portion of the oxygen-haemoglobin dissociation curve. The equivalent PO_2 would equate to approximately 135 mmHg which equates to an altitude of 2,200-2,500 m which is required to stimulate sufficient haemopoesis at rest to influence endurance performance (Levine et al, 1992). However, it has been demonstrated that the decrement of $\dot{V}O_{2max}$ measured in hypobaric hypoxia is directly proportional to $\dot{V}O_{2max}$ measured in normoxia (Shephard et al 1988). This would suggest that elite athletes are more prone to arterial hypoxaemia and may gain more benefit haematologically by training at lower altitudes in comparison to sedentary controls. This contention was supported by

Ingjer and Myhre (1992). They demonstrated that 3 wks of altitude training at 1900 m in elite cross country skiers was sufficient to elevate Hb by 5%, ($P < 0.05$) and decrease blood lactate concentration during a standardised sub-maximal test, despite no changes in $\dot{V}O_{2max}$. However, it should be noted that these authors did not measure their subjects' plasma volumes and their comments that the polycythaemia was independent of a haemoconcentration remains only speculative. The scarcity of training studies conducted at moderate altitudes of between 1500 to 2000 m in elite athletes does not allow for definitive conclusions to be made.

2.8.4 The Optimal Duration

Few data are available regarding the optimal duration an athlete should spend training at altitude. Based on subjective coaching opinion as opposed to objective scientific evidence, it would appear that 3 weeks are sufficient to gain a performance advantage following return to sea-level (Dick, 1992). However, the longer the duration of the hypoxic stimulus the greater the erythropoetic response and associated haematological adaptation (Schmidt et al 1993). Strategies of optimising the "erythropulse" at altitude were reviewed by Berglund (1992) who quantified the haematological changes during previous altitude training studies conducted between 1829 to 3048 m. He identified a "true" increase in Hb concentration of 1% per week, which was independent of a haemoconcentration. Therefore, assuming that the detraining response could be minimised and polycythaemia did not approach pathological values, the longer the athlete spends at altitude, the greater the potential benefit for endurance performance. However, future research should quantify the subsequent changes in blood flow during prolonged acclimatisation to altitude (Section 2.6.1).

Based on blood reinfusion studies, it would appear that a 4% increase in Hb concentration is the minimum stimulus required to increase $\dot{V}O_{2max}$ and sub-maximal exercise performance (Goforth et al 1982). This would suggest that a minimum of 4 weeks exposure to altitude hypoxia is required to increase the oxygen transport capacity of the blood. However, several altitude training studies have been conducted for significantly shorter durations, some as even as short as 7 d (Klausen et al 1991). Thus, the potential benefits of short durations at altitude are questionable for increasing aerobic capacity. Tables 2.9 and 2.10 indicate that few studies have been conducted with subjects who were optimally acclimatised to chronic hypoxia.

2.8.5 Iron Status During Altitude Training

A recent investigation using radio-iron labelling with ^{59}Fe (III)Cl_3 identified that 23 out of 45 male runners based at sea-level had low serum ferritin stores equivalent to <35 μg/L in comparison to a geometric mean value of 38 (+32/-17) μg/L (Nachtigall et al 1996). A sedentary age matched control group (n = 12) had significantly higher ($P < 0.001$) concentrations of serum ferritin [geometric mean value of 95μg/L, (=63/-38)]. It would appear that iron demand and mobilisation is further potentiated by hypoxia (Rejnafarje et al 1959 and Hannon et al 1969) such that endurance athletes training at altitude may be prone to iron deficiency. An investigation by Roberts et al. (1992) demonstrated a 50% decrease in serum ferritin which equated to a 340 mg decrease in stored iron during a 3 wk swimming training camp at 2,225 m. This occurred despite a dietary intake of iron equivalent to between 230 to 500% of the recommended daily allowance. Lack of this critical erythropoetic factor has been demonstrated to inhibit complete haematological adaptation at altitude (Stray-Gundersen et al 1992) and it has been suggested that endurance athletes should start oral iron supplementation equivalent to 200-300 mg daily 2-3 weeks prior to ascent and during 2-4 weeks at altitude (Berglund, 1992). Despite its importance, few studies have actually reported iron status of athletes during their hypoxic exposure. Sub-optimal iron stores may account for the vast majority of training studies which have failed to demonstrate increases in Hb concentration and endurance performance on return to sea-level following the hypoxic exposure. The differences in iron status may also characterise the highly individualised haematological response observed during altitude training (Ingjer et al 1992).

2.8.6 Interval Between Descent and Event

Is endurance performance affected by the timing of the descent to sea-level following a sojourn to altitude? The general consensus amongst top coaches would suggest that endurance performance is optimised 14 d following return to sea-level (Dick, 1992). There is emerging scientific evidence to support this claim. Suslov (1994) is the only investigator who has characterised the undulating nature of endurance performance following altitude training. His research was based on over 1000 competitive track results obtained from middle and long distance runners following different periods of altitude training, (1300 to 2500 m) and repeated sea-level $\dot{V}O_{2max}$ tests conducted after training at 1800 m. He identified a decrease in competition performance during the first 2 d at sea-level and the

first phase of enhanced work capacity occurring between days 3 to 7, followed by a decrease between days 8 to 10. Performance was shown to continue to improve between days 12 to 13, with the best results achieved on days 18 to 20. He also identified an additional upsurge in performance between days 36 to 48 post altitude. He failed to identify the physiological mechanisms responsible for this phenomenon.

Few studies have tested subjects on more than one occasion following return to sea-level. Asahina et al. (1966) and Faulkner et al. (1967) did not demonstrate any significant changes in $\dot{V}O_{2max}$ values following either 3 or 22 d at sea-level. Ingjer and Myhre (1992) demonstrated that after a group of elite cross country skiers had trained for 3 weeks at an altitude of 1900 m, sub-maximal blood lactate values were lower than pre-altitude values on day 1 but not day 14 at sea-level. The authors concluded that a 0.8 g/dl increase in Hb concentration measured on day one was responsible for the observed improvement in sub-maximal exercise. However, their failure to quantify plasma volume and blood flow changes questions the validity of their haematological findings. Svedenhag et al. (1991) studied a group of Swedish middle distance runners who trained for a period of 2 wks at altitude (2000 m) and were tested following 6 and 12 d on return to sea-level. They did not identify any significant changes in $\dot{V}O_{2max}$, maximal oxygen deficit and sub-maximal blood lactate values in comparison to pre-altitude values or between days 6 and 12 at sea-level. However, they showed a significant reduction in heart rate, Borg ratings of perceived exertion and plasma ammonia concentration during a standardised sub-maximal treadmill test which was more apparent following 12 d at sea-level.

The physiological mechanisms responsible for these subtle changes in performance at sea-level remain elusive. Intermittent altitude training has been shown to increase the hypoxic ventilatory response (HVR) in a group of sedentary subjects, whereas an equivalent training program at sea-level had no effect (Benoit et al 1992 and Levine et al 1992). Chronic altitude training may potentiate the HVR due to an increased peripheral chemoreceptor sensitivity which would subsequently increase the work performed by the respiratory muscles. This has not been quantified in the elite athlete but may be implicated in the performance decrements shortly after return to sea-level. Plasma volume has been demonstrated to decrease by 25% during chronic exposure to hypobaric hypoxia (Young et al 1988) and may take up to 2 months at altitude before it returns to pre-altitude values (Reynafarje et al 1959). Following return to sea-level, this may remain depressed for 6

days (Dill et al 1974) which may also negatively affect performance. The negative impact of jet lag on exercise performance (Reilly et al 1994) cannot be ignored if altitude training requires significant travelling time.

2.9 POTENTIAL DANGERS OF ALTITUDE TRAINING

Despite the continued popularity of altitude training, little is known regarding the potentially less favourable physiological responses to environmental hypoxia. The fitness of the subject at altitude is challenged by a number of factors which include; decreased absolute training intensity (Stine et al 1992), decreased plasma volume (Young et al 1988), depression of haemopoesis and increased haemolysis (Szygula, 1990), increased sympathetically mediated glycogen depletion (Young, 1990) and increased respiratory muscle work following return to sea-level (Levine et al 1992 and Benoit et al 1992).

There are also important health implications that need to be considered with the possible risk of developing more serious medical complications at altitude. These include: acute mountain sickness, pulmonary oedema, cardiac arrhythmias and cerebral hypoxia (Shephard, 1992). Changes in immune function at altitude have also been largely ignored, despite accumulating evidence of hypoxia-mediated immunosupression (Meehan, 1987). A summary of the possible advantages and disadvantages of altitude training and their approximate time constants are presented in Table 2.12.

Table 2.12 Physiological Changes During Altitude Acclimatisation in Native Lowlanders; Time Course and Theoretical Implications for Exercise Performance at Sea-Level

Physiological Advantages	Response Time	Physiological Disadvantages	Response Time
Increased FFA mobilisation	Weeks	Increased ventilation	Immediate
Increased Hb	Days	Decreased cardiac	Days
Increased capillarity	Months/Years?	output	
Increased oxidative	Weeks	Decreased blood flow	Days
enzyme activity		Immunosupression	Immediate?/Days
Increased mitochondrial	Weeks	Increased oxidative	Immediate?/Days?
volume		stress and tissue damage	
		Increased dehydration	Immediate
		Jet lag (only if time zones	Immediate
		are crossed)	
		Decreased training	Immediate
		intensity	
		Acute mountain sickness	Days
		Sunburn due to increased	Immediate
		ultra-violet B (290-320nm)	
		Catecholamine-mediated	Days-weeks
		glycogen depletion	
		Increased haemolysis	Weeks

2.9.1 Hypoxia and Immune Function

Changes in total leukocyte count, granulocyte, monocyte, lymphocyte, natural killer cell count, total T cell count, helper : suppression cell ratio, cell proliferation in response to mitogens and serum immunoglobin levels, have all been implicated in some form of immunosuppression which may subsequently cause underperformance in the athlete at sea-level (Shephard et al 1994). The additive stress of a reduction in the inspiratory PO_2, in conjunction with the extensive training loads employed by athletes at altitude, may explain why some investigators have reported physiological evidence for a less favourable

modulation of *in vivo* immune function during acute and chronic exposure to hypobaric hypoxia (Rusko et al 1996; Uchakin et al 1995; Simon-Schnass, 1994 and Meehan et al 1988). Human studies have demonstrated that chronic exposure to hypobaric hypoxia results in a suppression of cell-mediated immunity, whereas B cell function remains unimpaired (Meehan, 1987). Animal studies have further demonstrated that murine host defences against bacterial pathogens are also impaired in hypoxia. The contributory immunomodulatory role of endogenous glucocorticoids and neuropeptides, which are increased at altitude, may contribute to the observed alterations in immune competence. The implications of the immunosupressive influence of hypobaric hypoxia for endurance performance warrants further investigation in order to elucidate potential mechanisms which may modulate performance following return to sea-level.

2.9.2 Reactive Oxygen Species at Altitude

Free radical production has been shown to increase during physical exercise (Davies et al 1982 and Jackson et al 1985), the implications of which are currently the subject of much interest in exercise biochemistry. Whilst oxygen-centred free radicals have been implicated in the regulation of blood flow (Rhoades et al 1990), neural transmission (Aizenman et al (1990) gene regulation (Shibanuma et al 1990) and mitochondrial biogenesis (Salo et al 1991) there is an accumulating body of evidence which *associates* excess free radical production with tissue damage (Jackson, 1996).

Animal studies have suggested that oxidative injury mediated by free radicals is further increased during chronic exposure to environmental hypoxia (Biselli et al 1992 and Radak et al 1994). Using thiobarbituric reactive substances (TBARS) as an indirect marker of lipid peroxidation, the latter investigators demonstrated a marked increase in free radicle activity in rat soleus muscle at 4,000 m (Radak et al 1994). They attributed these changes to a decrease in the activity of the antioxidant enzyme, mitochondrial superoxide dismutase.

A limited number of human studies have also demonstrated increases in free radical activity at altitude (Nagawa et al 1968; Simon-Schnass, 1994 and Biselli et al 1992). Simon-Schnass (1994) have identified significant increases in indirect indices of free radical mediated lipid peroxidation which included increased pentane excretion and TBARS, decreased erythrocyte filterability and increased leukocyte and granulocyte counts.

Daily supplementation with an antioxidant such as tocopherol (vitamin E) equivalent to 300-400 mg has been demonstrated to improve endurance performance, by theoretically limiting tissue damage (Nagawa et al 1968 and Simon-Schnass, 1994).

An accelerated production of the highly toxic hydroxyl radical (•OH) may occur as a consequence of an increased production of free iron derived from altitude and training-induced destruction of red blood cells (Szygula et al 1990). Thus, it would seem that environmental hypoxia significantly increases oxidative stress which has been demonstrated to negatively influence energy metabolism and membrane integrity.

2.10 SUMMARY AND DIRECTIONS FOR FUTURE RESEARCH

Physiological acclimatisation to a chronically reduced P_IO_2 is a prerequisite to achieve optimal physical performance in environmental hypoxia. However, scientific evidence to support the claim that either continuous or intermittent hypoxic training will enhance sea-level performance remains at present equivocal. Future research should focus on methodological technicalities that optimise the balance between the favourable and less favourable responses to hypoxia and potential mediators of performance following return to sea-level. Preliminary evidence which has demonstrated that the additive stress of hypobaric hypoxia may provoke an adverse immune response and further potentiate free radical mediated oxidative injury has important implications which, if confirmed by scientific rigour, would present a threat to both the fitness and health of the elite competitor.

2.11 EXPERIMENTAL AIMS AND NULL HYPOTHESES (H_O)

In light of the previous discussion, there were four major experimental aims to the research described in the present thesis. These are summarised below:

Aim [1]: *To evaluate the effects of hypobaric hypoxia on physiological indices of exercise performance in a cohort of elite distance runners.*

Aim [2] *To determine the implications of chronic exposure to hypobaric hypoxia on physiological correlates of exercise performance following return to sea-level.*

Aim [3] *To examine factors that are potentially implicated in the modulation of exercise performance at altitude and following return to sea-level.*

Aim [4] *To investigate the potentially adverse physiological responses to hypobaric hypoxia and subsequent implications for the fitness and health of the elite competitor.*

These experimental aims were fulfilled during two separate training studies that were conducted over a twelve month period:

Study [1] New Mexico altitude training camp (USA):
Determination of the effects of 4 weeks of moderate altitude training (1,500-2,000 m) on physiological indices of *submaximal* and *supramaximal* running performance at altitude and following 21 days return to sea-level.

Study [2] Krugersdorp altitude training camp (S.Africa):
Determination of the effects of 4 weeks of moderate altitude training (1,640 m) on physiological indices of *maximal* and *supramaximal* running performance at altitude and following 10 and 20 days return to sea-level.

Six null hypotheses were thus formulated and examined throughout the thesis. These are outlined below:

Null Hypothesis [1]: *Hypobaric hypoxia does not affect the physiological response during and following recovery from maximal exercise.*

Null Hypothesis [2]: *Hypobaric hypoxia does not affect physiological responses during and following recovery from supramaximal exercise.*

Null Hypothesis [3]: *Four weeks of continuous exposure to hypobaric hypoxia does not improve physiological indices of submaximal performance three weeks following return to sea-level.*

Null Hypothesis [4]: *Physiological performance during and following recovery from maximal exercise at sea-level are not improved following four weeks of continuous exposure to hypobaric hypoxia.*

Null Hypothesis [5]: *Four weeks of continuous exposure to hypobaric hypoxia does not potentiate physiological indices of supramaximal exercise performance following return to sea-level.*

Null Hypothesis [6]: *Immune function is not altered by chronic hypobaric hypoxia.*

CHAPTER 3

THEORETICAL AND METHODOLOGICAL BACKGROUND

3.1 INTRODUCTION

This chapter outlines the analytical techniques and scientific theory involved during the investigations. Two experimental investigations were preceded by a series of quality control studies and two pilot studies. A detailed discussion of the experimental methods is outlined in Chapters 4 and 5.

3.2 SELECTION OF SUBJECTS

Subjects were selected from a pool of elite middle to long distance runners who were based at either Loughborough or London, UK. Recruitment took place following discussion with the Great Britain National Endurance Events Coach (3 km to 10 km).

The criteria for participation in this research was that each subject had gained International honours in either track, road or cross country running disciplines. Potential subjects were excluded from participation if they were not in good health. Each subject was informed about the requirements of this research and subsequently completed a medical questionnaire before they gave their written consent to participate (Appendix A). They were informed that they would receive a detailed report summarising the major physiological changes and subsequent implications for exercise performance throughout the studies (Appendix B). It was considered that this strategy would maximise compliance.

Twenty seven male and 11 females (n = 38) who were born and raised at or near sea-level volunteered for two altitude training studies that were conducted over a fourteen month period. Sixteen male and 7 females (n = 23) participated in Study 1 and 22 males and 7 females (n = 29) participated in Study 2. Eleven males and 3 females (n = 14) were involved in both studies.

3.3 PRE-EXPERIMENTAL DATA COLLECTION

3.3.1 Dietary Analysis

Two separate dietary analyses were performed for studies 1 and 2 respectively due to financial constraints. The funding for dietary analysis 2 was provided by the British Olympic Association.

[Dietary analysis 1]: For the 7 days prior to the start of study 1, each subject recorded the quantity and type of food and beverage ingested. This data was analysed using a commercialised software package (Nutri-Check, Health Options Limited, Nottingham, UK).

[Dietary analysis 2]: Each subject recorded the quantity and type of food and beverage ingested for a 3 day period at sea-level prior to the start of Study 2. This information was analysed using a computer software package (Compeat, UK).

All subjects were instructed to refrain from alcohol consumption and physical training for 48 h prior to physiological testing. Due to the nature of the social environment on a training camp, it is always difficult to guarantee absolute abstension. However, any deviation from the recommendations was considered minimal due to the conscientious approach that these subjects have to training.

3.3.2 Training Load and Intensity

Accurate quantification of training load was considered an integral part of this research, a variable which has been largely ignored by previous investigators. Each subject logged the amount (km completed each week) and intensity (determined using a Polar Vantage NV™ heart rate monitor) of physical exercise performed during a typical week of training at either sea-level and/or at the respective altitude training camps (Appendix C).

Changes in barometric pressure during the training sessions at altitude were recorded with a portable aneroid barometer (Model 943, Casio, Japan). This instrument was calibrated at sea-level using a Fortin barometer.

3.3.3 Treadmill Calibration

Laboratory tests were administered on two motorised treadmills (Powerjog GXC200, Birmingham, UK and Woodway, Switzerland). Each treadmill was calibrated prior to testing at a range of velocities (5 $km.hr^{-1}$ - 24 $km.hr^{-1}$) and gradients (0.5% - 10%) that were to be encountered during the study. Treadmill velocity was checked manually by measuring the length of the treadmill belt and recording the time taken to complete 50 revolutions. These calculations were verified automatically using a tachometer (Compact, CT2, UK). Treadmill gradient (%) was validated using a spirit level and set square. The % gradient was calculated as the sine of the angle (sin θ), in which sin θ equalled the vertical rise over the hypotenuse.

3.4 ENVIRONMENTAL CONDITIONS

Control of ambient conditions is an important component of any experiment, when one considers the physiological implications of thermoregulation on performance (Sutton, 1994).

3.4.1 Laboratory Conditions

Experimental data were collected in 3 different laboratories during studies 1 and 2:

[1] British Olympic Medical Centre, UK.
[2] Loughborough University, UK.
[3] Witwatersrand Medical University, S.Africa.

Ambient temperature and relative humidity (%) were continuously checked using a whirling hygrometer (Casella, London, UK). With the exception of the physiology laboratory at Loughborough University, ambient temperature was controlled using a mounted air conditioning unit (Model RAZ-1003CHE8, Toshiba, Japan) which maintained the laboratory at 21 $\pm$ 1°C. Ambient temperature at Loughborough University ranged from 18.8°C to 23.0°C during the study.

3.4.2 Track Conditions

A standardised track session was conducted on a tartan track at the following venues:

[1] Loughborough University, UK.

[2] Roger Bannister Stadium, London, UK.

[3] Albuquerque, New Mexico, USA.

[4] Krugersdorp, S.Africa.

Ambient temperature and relative humidity were recorded during each track session. Retrospective recordings of wind velocity ($m.sec^{-1}$) at the respective venues were made based on data provided by the Meteorological Office, London Weather Centre, UK, New Mexico Meteorlogical Centre, USA and Johannesburg Meteorlogical Centre, S.Africa.

3.5 ANTHROPOMETRIC MEASUREMENTS

3.5.1 Body Mass and Height

Each subject was instructed to wear a swimming costume and remove footwear prior to the measurement of body mass using a balanced weighing scales (Seca, Cardiokinetics, Salford, UK) and height measured with a stadiometer (Seca, Cardiokinetics, Salford, UK). The weighing scales were calibrated prior to each "weigh in" with a 5 kg free weight. The accuracy of the stadiometer was checked with a tape measure.

3.5.2 Body Fat

Harpenden skinfold calipers (John Bull, British Indicators LTD, Bedfordshire, UK) with a constant spring pressure of $10g/mm^2$ were accurately calibrated with a Vernier scale and used to measure skinfold thickness at the biceps, triceps, subscapular and suprailiac sites on the left hand side of the subject's body. Three repeated measurements were obtained from each skinfold site and the sum of the mean values was used to indirectly calculate body fat using a modification of the original regression equations developed by Durnin and Womersley (1974), (B.Carpenter, unpublished observations, British Olympic Medical Centre, UK). A brief discussion of this procedure is outlined in Appendix D.

The coefficient of variation (CV) of repeated skinfold measurements by the same trained observer has been estimated at 5% (Durnin and Womersley, 1974 and Lohman, 1981) and has been demonstrated to correlate highly (r = 0.83 - 0.89) with the hydrostatic weighing technique (Durnin and Womersley, 1974).

3.6 HAEMATOLOGICAL MEASUREMENTS

3.6.1 Blood Sampling

All blood samples were collected at the same time of day by the same investigator (07:30 to 09:30 hr) in an attempt to control for biological variation (Reilly, 1994) and minimise inter-subject analytical variation (Appendix E). Diet has also been shown to exert a modulating effect on several blood borne metabolites, in particular plasma lipids and lipoproteins (Pronk, 1993). Therefore, all venous blood samples were taken following a 12 h overnight fast. Arterialised capillary blood samples were obtained 3 h post prandial. The inclusion of female subjects in these investigations posed a particular problem during the metabolic analysis due to the contaminating effects of female androgens (Appendix F). The stage of the menstrual cycle was noted at the time of testing and considered during the overall analysis of results. Blood was sampled from either the left earlobe (arterialised capillary blood) or from an antecubital vein (venous blood) 30 min after the subject had assumed a seated position to control for plasma volume shifts (Pronk, 1993).

3.6.1.1 Collection of Arterialised Capillary Blood

The left earlobe was warmed with a commercial hairdryer and cleaned with swabs saturated with 70% v/v Isopropyl alcohol (Medi Swab, Smith and Nephew, UK). Prior to exercise, the sample site was punctured using a sterile stainless steel lancet (Lance, Sheffield, UK) and excess blood was wiped clean with a tissue (Kimwipes^{R}, Kimberley Clark, UK). To obtain a blood sample, a gentle pressure was applied to the earlobe using the thumb and index finger. It was necessary to rhythmically "milk" the sample site following exercise to increase bloodflow to counteract peripheral vasoconstriction. Whilst milking has been suggested to dilute the blood sample by introducing extravascular fluid into the sample, evidence suggests that this is not the case (Godsen et al 1991 and Bailey and Davies, unpublished data). Care was taken to remove excess sweat from the earlobe prior to blood sampling. Contamination by sweat has been demonstrated to confound haematological measurements, in particular concentrations of whole blood lactate ($[La^{-}]_B$). Sweat lactate levels have been shown to be considerably greater than blood lactate levels (Pilardeau et al 1988).

3.6.1.2 Collection of Venous Blood

Each subject assumed a seated position and a tourniquet was secured to the right arm with the minimum constriction required to obtain a blood sample (Bachorik, 1982). Four venous blood samples (~ 40 ml) were obtained from an antecubital forearm vein and collected into Vacutainer® tubes (Becton Dickinson, Rutherford, NJ, USA) that were immediately placed on ice.

3.6.2 Procedures for the Measurement of Iron Status

Whilst several studies have identified that elite athletes training at sea-level suffer from the early stages of iron defficiency (iron depletion), exercise performance does not appear to be affected unless the defficiency progresses to anaemia (Clarkson, 1990). However, it has been suggested that the incidence of iron deficient anaemia may increase at altitude (Berglund, 1992). Suboptimal iron stores combined with a marked increase in iron demand and mobilisation (Reynafarje et al 1959, 1961) have been shown to inhibit complete haematological adaptation at altitude (Hannon et al 1967, 1969 and Stray-Gundersen et al 1992). A decrease in iron stores due to haemolysis has also been demonstrated at altitude due to an increased red blood cell fragility; administration of polyunsaturated fatty acids has been shown to counteract this (Bigard et al 1991). Oxidative stress mediated by free radical activity ($R^{\bullet}$) is also implicated in iron loss due to the irreversible damage to erythrocyte lipids and proteins. The major cause of $R^{\bullet}$ generation is through auto-oxidation of oxyhaemoglobin to met-haemoglobin which ultimately generates superoxide (Smith, 1995). A combination of physical exercise and environmental hypoxia could theoretically potentiate this process (Section 2.11.2). Samaja et al. (1993) provided indirect support for this hypothesis by demonstrating that erythrocytes age at a faster rate at 5,050 m. There is at present overwhelming evidence to suggest that oxidative damage may be the primary mechanisms by which the erythrocyte ages (Clark, 1988).

The iron status of each subject was therefore determined prior to the altitude sojourn in an attempt to ensure optimal haematological adaptation. Whilst an increased iron absorption constitutes what is considered to be the most sensitive parameter of iron deficiency (Nachtigall et al 1996) it's commercial availability is limited. Thus, the combined measurements of haemoglobin, serum ferritin, serum vitamin B_{12}, red cell folate, plasma

iron and transferrin were used to assess iron status. A brief review of the analytical procedures involved is outlined in the following sections.

3.6.2.1 Haemoglobin (Hb)

The iron content within the human body varies between 2 to 5g (Huebers and Finch, 1987), of which approximately two thirds are contained within Hb (Cook, 1994). The concentration of Hb in whole blood was measured photometrically according to the procedures described by Vanzetti (1966). This procedure has been validated against the classical hemiglobincyanide (HiCN) method. The HemoCue® method involves the haemolysis of erythrocytes with sodium deoxycholate which subsequently releases Hb. Sodium nitrite converts Hb to methaemoglobin which, when combined with sodium azide forms azidemethaemoglobin. The absorbance is subsequently measured at two wavelengths (570 and 880 mm).

Following calibration with an optical interference filter (Hb = 13.7±0.3 g/dl) approximately 10µl of arterialised capillary blood from the left earlobe was collected in a cuvette (HemoCue® B-Hemoglobin, Sheffield, UK). This was inserted into the photometer (HemoCue® B-Hemoglobin, Sheffield, UK) and a digital readout was presented within 15 to 45 sec. Concentrations of Hb were measured in triplicate and the mean value, expressed in g/dl, was used during analysis.

3.6.2.2 Packed Cell Volume (PCV)

Arterialised capillary blood was obtained from an earlobe blood sample and collected into a heparinised capillary tube which conformed to British Standard BS 4316 (75mm, 59µl, Hawksley and Sons Limited, Sussex, UK). Care was taken to ensure that there were no air bubbles present in the sample. The distal end of the tube was sealed with cristaseal (Hawksley and Sons Limited, Sussex, UK) and inserted into a Micro Haematocrit Centrifuge (Hawksley and Sons Limited, Sussex, UK) with the sealed end facing outwards. The blood sample was immediately spun at 11, 800 revolutions per minute (RPM) for 5 minutes and the measured length of the column of packed erythrocytes was measured using a Hawksley Micro Haematocrit Reader (Hawksley and Sons Limited, Sussex, UK). This value expressed in litres per litre of whole blood (L/L) was corrected by 1.5% for plasma trapped between the erythrocytes (Dacie et al 1968 and Harrison, 1985). Triplicate

capillary blood samples were analysed for PCV and the mean value was used in the overall analysis.

3.6.2.3 Serum Ferritin

Ferritin and haemosiderin (ferritin aggregates) constitute the major source of the body's intracellular iron storage proteins. These are located in the bone marrow, reticuloendothelial cells and the liver (Dallmann et al 1993). The minute concentration of ferritin secreted into the circulation is routinely used to assess total iron stores (Nachtigall et al 1996). It has been estimated that 1μg of ferritin per litre of serum represents approximately 8 mg of storage iron (Birgegard et al 1977). Using radio-iron labelling (^{59}Fe) and liver iron quantification techniques, a recent study has demonstrated that serum ferritin values < 12 μg/L represents completely and < 35 μg/L represents partly depleted iron stores (Nachtigall et al 1996). Ferritin is an acute phase protein and falsely elevated serum ferritin concentrations can occur in response to strenuous physical exercise and thus mask iron deficiency and haemolysis in athletes (Witte et al 1991). It has even been suggested that elevated serum ferritin be used as an indicator of overtraining (O'Toole et al 1989).

Serum ferritin was measured using a solid phase enzyme immunoassay (Roche Cobas Core Analyser). A summary of the biological principles and procedures of the measurement are outlined below:

The measurement of serum ferritin is based on Microparticle Enzyme Immunoassay (MEIA) technology. Ferritin reagents and the sample were pipetted in the following sequence.

Sampling Centre:

- The sample and ferritin reagents were pipetted by a sampling probe into various wells of a reaction vessel (RV).
- The sample was pipetted into one well of the RV.
- Anti-Ferritin (mouse, monoclonal) Coated Microparticles in TRIS buffer with protein stabilisers, Anti-Ferritin (Rabbit) Alkaline Phoshpatase Conjugate in TRIS buffer with protein stabilisers, Specimen Diluent containing TRIS buffer with surfactant and protein

stabilisers and TRIS Buffer (with 0.3 M sodium chloride) were pipetted into another well of the RV.

The RV was immediately transferred to the Processing Centre where further pipetting was performed by the Processing Probe:

Processing Centre:

- An aliquot of the Specimen Diluent, Conjugate, Microparticles and TRIS Buffer mixture was pipetted and mixed with the sample.
- The ferritin, enzyme-labelled antibody and microparticles bind forming an antibody-antigen-antibody complex.
- An aliquot of the reaction mixture containing the antibody-antigen-antibody complex bound to the microparticles was transferred to the matrix cell where the microparticles bind irreversibly to the glass fibre matrix.
- The matrix cell was washed to remove any unbound material.
- The substrate, 4-methylumbeliferyl phosphate was added to the matrix cell and the fluorescent product was measured by the MEIA optical assembly

3.6.2.4 Transferrin

The measurement of the soluble transferrin receptor concentration in plasma is a useful index of erythropoiesis in athletes (Cook et al 1993). The combination of ferritin, soluble transferrin receptor concentrations and blood reticulocyte counts can discriminate between the 4 major stages of anaemia which include iron deficiency, hypoproliferative anaemia, maturation disorders and haemolytic anaemia (Cook et al 1993). Transferrin sequesters iron from catabolised haemoglobin or tissue stores and delivers it to proliferating erythrocyte precursors for haemoglobin synthesis (Smith, 1995).

Transferrin concentration was determined by rate immunonephelometry using a Beckman Array Analyser.

3.6.2.5 Serum Iron

Serum iron was measured via colometric dye binding using dry slide reagents. The analysis was performed with an Ortho Clinical Diagnostics Vitros 750 analyser. A summary of the analytical procedures is outlined below:

- A 10µl drop of patient sample was deposited onto a *Vitros* Fe Slide which is a multilayered element coated on a polyester support. The sample is evenly distributed by the spreading layer to the underlying layers. Iron (as ferric ion) is removed from transferrin at acidic pH and migrates to the reducing layer where ascorbic acid reduces iron to the ferrous form. The ferrous iron is bound to N-(4-(2,4-bis(1,1-dimethylpropyl)phenoxy)butyl)-5-methoxy-6(2,3,6,7-tetrahydro-8-1H,5H-benzoquinolizin-9-yl)azo)-3-pyridine sulfonamide to form a coloured complex in the reagent layer. The slide was incubated at 37°C and two reflection density measurements at 600 nm were conducted at one and five minutes. The rate of change in reflection density is proportional to the iron concentration in the sample. The reaction sequence is summarised below:

$$\text{transferrin-Fe}^{+3} \xrightarrow{\text{pH 4.0}} \text{transferrin} + \text{Fe}^{+3}$$

$$\text{Fe}^{+3} + \text{ascorbic acid} \longrightarrow \text{Fe}^{+2}$$

$$\text{Fe}^{+2} + \text{dye} \longrightarrow \text{Fe}^{+2}\text{ - dye (colored complex)}$$

3.6.2.6 Red Cell Folate and Plasma Vitamin B_{12}

These two B-complex vitamins must be available in sufficient quantities to ensure a normal rate of erythropoiesis as they are essential for DNA synthesis (Marieb, 1989). These were measured by radioimmunoassay using a BioRad Quantaphase Kit, the principles of which are discussed below:

A serum sample was combined with vitamin B_{12} (^{67}Co) and folate (^{125}I) in a solution containing dithiothreitol (DTT) and cyanide. The mixture was boiled to inactivate endogenous binding proteins and to convert the various forms of vitamin B_{12} to cyanocobalamin. The reduced folate and its analogs were stabilised by DTT during the heating. The mixture was subsequently cooled and then combined with immobilised, affinity-purified porcine intrinsic factor and folate binding protein. This addition adjusts and buffers the pH of the reaction mixture to 9.35. The reaction mixture was then incubated for 1 hour at room temperature.

During incubation, the endogenous and labelled vitamins compete for the limited number of binding sites based on their relative concentrations. The reaction mixture was then

centrifuged and decanted. Labelled and unlabelled vitamins binding to the immobilised binding proteins were concentrated at the bottom of the tube in the form of a pellet. The unbound vitamins in the supernatant were discarded and the radioactivity associated with the pellet was counted. Standard curves were prepared using vitamin B_{12} and folate standards in a human serum albumin base. The concentrations of the vitamin B_{12} and folate in the serum sample were determined from the standard curves

3.6.3 Procedures for the Measurement of Plasma Lipids and Lipoproteins

Triglycerides and other lipids are insoluble in plasma and circulate as particles known as lipoproteins (Ball and Mann, 1994). These lipoproteins are large, globular particles that contain an oily core of nonpolar lipid (cholesteryl ester or triglycerides) surrounded by a polar coat of phospholipids, free (unesterified) cholesterol and apolipoproteins (Brown and Goldstein, 1990).

Lipoproteins are mostly classified on the basis of their gravitational density; six classes are present, which differ according to their size, density, in the relative proportions of triglycerides and cholesterol esters in the core and in the nature of the apolipoproteins on their surface (Pronk, 1993). These classes include:

1. Chylomicrons
2. Chylomicron remnants
3. Very low density lipoproteins (VLDL)
4. Intermediate density lipoproteins (IDL)
5. Low density lipoproteins (LDL)
6. High density lipoproteins (HDL)

Physical characteristics of the major lipoprotein classes are outlined in Table 3.1.

Table 3.1 Physical Characteristics of the Major Lipoproteins in Man (Modified from Haskell, 1984)

Physical Characteristic	Lipoprotein Class: HDL	LDL	VLDL	Chylomicron
Ultracentrifugal density (g/ml)	1.21 - 1.063	1.063 - 1.006	1.006 - 0.95	<0.95
Flotation rate (Svedborg units)	.....	0 - 20	20 - 400	>400
Electrophoretic mobility	Alpha	Beta	Prebeta	Origin
Molecular weight	$2 - 4 \times 10^5$	$2.1 - 2.6 \times 10^6$	$5 - 10 \times 10^6$	$10^3 - 10 \times 10^5$
Size (Å)	50 - 150	215 - 220	300 - 800	$400 - 10^4$
Lipid content (% of total lipid)	~ 50	~ 75	~ 90	~ 98
Triglyceride	4 - 10	10	50 - 70	85 - 95
Cholesterol	25 - 35	45 - 60	10 - 20	4 - 12
Phospholipid	30 - 50	20 - 30	10 - 20	4 - 12
Protein (% dry weight)	45 - 55	20 - 25	10 - 15	0.5 - 2.5
Apolipoprotein Class				
Major	A-I, A-II	B	B, C-III, E	B,C-I, C-II, C-III
Minor	C-III, B, C-1 C-II, E	C-I, C-II	A-I, A-II, D-I C-II	A-I, A-II, E
Production site				
Major	Intestine	Plasma	Liver	Intestine
Minor	Liver	.....	Intestine	.....

3.6.3.1 Total Cholesterol and Triglyceride

Total cholesterol (TC) and triglyceride (Tg) concentrations were determined by routine enzymatic techniques using an Olympus AU5200 automated analyser and Olympus reagents.

3.6.3.2 High Density Lipoprotein Cholesterol and Low Density Cholesterol

The cholesterol content of the high density lipoprotein (HDL-C) was assayed enzymatically after chemical precipitation of other lipoproteins from serum with dextran sulphate and magnesium (Warnick et al 1982).

Low density cholesterol (LDL-C) was derived according to the Friedewald et al. (1972) formula, given by:

$$\text{LDL-C } (\text{mmol.L}^{-1}) = \text{Total cholesterol} - \frac{\text{triglycerides}}{2.2} - \text{HDL}$$

3.6.3.3 Apolipoproteins and Lipoprotein (a)

Apolipoprotein A, apolipoprotein B and lipoprotein (a) [Lp(a)] were measured by rate immunonephelometry using a Beckmann ARRAY analyser using Beckmann reagents.

3.6.4 Plasma Glutamine

Glutamine is a neutral amino acid which is produced predominantly by skeletal muscle (Newsholme and Leech, 1983) but also by the lungs (Ardawi et al 1990), liver (Haussinger et al 1989), brain (Souba et al 1992) and possibly the adipose tissue (Frayn et al 1991). At an intracellular level, the two principal enzymes involved in glutamine metabolism are glutaminase which catalyses glutamine hydrolysis to glutamate and ammonia and glutamine synthetase which catalyses glutamine synthesis from ammonia and glutamate (Salway, 1995). These maintain "normal" resting concentrations of glutamine in human skeletal muscle which have been estimated at 20mM/l of intracellular water with a corresponding plasma concentration of 0.6mM (Bergstrom et al 1974).

Recent research would suggest that glutamine is a highly versatile amino acid required for the optimal functioning of several important homeostatic functions (Rowbottom et al 1996). These include transfer of nitrogen between organs and detoxification of ammonia (Newsholme et al 1983), maintenance of the acid base balance during acidosis (Damian et al 1970), serving as a nitrogen precursor for the synthesis of nucleotides (Krebs, 1980), a fuel for gut mucosal cells (Windmueller et al 1974) and optimal functioning of cells of the immune system (Ardawi et al 1985). The latter investigators demonstrated that glutamine is a key substrate required at high rates for lymphocytes and macrophages and any physiological decrease in the circulating plasma glutamine concentrations would represent

an impairment in the body's ability to defend itself against an antigenic challenge with resultant susceptibility to infection. In addition to this, glutamine is an important precursor for γ-aminobutyric acid synthesis which acts as an inhibitory neurotransmitter. A decreased inhibition of the neuronal firing rate may be a contributory factor related to altering perceived exertion and premature fatigue (Banister et al 1985). An overview of the analytical procedures involved during the measurement of plasma glutamine is discussed in the following sections.

3.6.4.1 Deproteinisation of the Sample

Blood samples were deproteinised according to the methods described below (Bernt and Bergmeyer, 1974):

[1] Frozen plasma samples of heparinised venous blood were thawed and centrifuged for 30 s at 1,400 rpm in a micro-centrifuge (Heraeus Biofuge A) to remove fatty deposits.

[2] Two aliquots of plasma (300µl) were placed in separate Eppendorf tubes (1.5 ml) and 300µl of 10% perchloric acid (PCA) was added to each. These were immediately centrifuged as glutamine has been demonstrated to degrade rapidly in acidic conditions (Krebs, 1933) and two additional Eppendorf tubes were prepared as follows:

15µl pH Universal indicator to determine changes in pH
50µl 0.5mM triethanolamine (TEA buffer)
100µl of KOH

[3] Exactly 460µl of supernatant was added to each tube, vortexed and the pH was manually adjusted to 7.0 to 7.5 (lightish green) by adding 20% KOH and/or supernatant and the quantity recorded.

[4] The deproteinised samples were subsequently aspirated with a plastic pipette and stored in new Eppendorf tubes at -20°C.

3.6.4.2 Enzymatic Assay

A slight modification of the enzymatic assay developed by Windmueller and Spaeth (1974) was used to determine plasma glutamine concentrations. The asparaginase assay was chosen in preference to the glutaminase assay (Lund, 1974) due to "suspect" results that were obtained if samples were deproteinised elsewhere and subsequently assayed in the laboratory (personal communication, Miss L.Castell, Department of Biochemistry, University of Oxford, UK). Asparaginase was dialysed for 24 h against two changes of KH_2PO_4 buffer (80 mM, pH 6.6) prior to use. The reaction mixture (1,060 µl) contained the following:

Reagent	Final Concentration (Units)
KH_2PO_4	45 mM
NADH	172 µM
α-ketoglutarate	3.6 mM
Glycerol*	8%
BSA*	0.05%
Glutamate dehydrogenase	0.5 mg
Asparaginase	20 units

* - protected assay enzymes from denaturation.

The biochemical principles involved for the measurement of plasma glutamine concentration are based on the following enzymatic reactions:

$$[1]\ \text{Glutamine} \xrightarrow{H_2O + \text{Asparaginase}} \text{Glutamate}$$

$$[2]\ \text{Glutamate} \xrightarrow[\text{Glutamate dehydrogenase}]{H_2O;\ NAD^+ \rightarrow NADH + H^+} \alpha\text{-ketoglutarate} + NH_4^-$$

[1] Glutamine is hydrolysed with asparaginase to produce glutamate.

[2] The glutamate concentration is determined indirectly by the hydrolysis of glutamate to α-ketoglutarate with glutamate dehydrogenase. Oxidised NAD^+ is converted to its reduced

form, NADH + H^+ during this reaction which is detected spectrophotometrically at 340 nm (Gilford, Stasar III, UK). The concentration of plasma glutamine is calculated based on the principle that the conversion of glutamine to glutamate is on a molar to molar basis.

There are several other analytical techniques which have been employed to measure plasma glutamine concentration in humans (Rowbottom et al, 1996). It is clear from the data contained in Table 3.2 that the methodological procedure should be considered when comparing data from separate scientific investigations.

Table 3.2 Mean Values for Resting Plasma Glutamine Concentration in Humans: A Comparison of Analytical Methods (Rowbottom et al, 1996)

Method	Plasma Glutamine (μmol/L)
Enzyme*	614 ± 27
HPLC	594 ± 18
Bioassay (E.*coli*)	1021 ± 47

* - Method employed in the present investigations
HPLC - High performance liquid chromatography

3.6.5 Measurement of Whole Blood Lactate ($[La^-]_B$)

Approximately 50μl of arterialised capillary blood was collected into a capillary tube (Analox). Each capillary tube was lined with heparin, fluoride and nitrite to stabilise blood lactate concentrations. The capillary tube was immediately placed onto a battery operated mixer for 4 minutes. Research in our laboratory has identified that the mixing period needs to be controlled prior to the analysis of $[La^-]_B$ (Bailey and Davies, unpublished data). Each sample was rotated approximately 180° every 5 sec. This ensured adequate mixing of whole blood with the biochemical agents and prevented coagulation prior to analysis.

The concentration of $[La^-]_B$ was measured using an automated electrochemical analyser (Analox PGM7 Champion, London, UK). Fresh buffer was added to L-lactate:oxygen oxidoreductase (LOD) at an ambient temperature of 21°C and entrained into the analyser. Injection of a sample into the cuvette activates an oxidation-reduction reaction catalysed by L-lactate:oxygen oxidoreductase (LOD) at a pH of 6.5. The maximum rate of oxygen

consumption during the reaction is directly related to the concentration of lactate in the sample.

$$\text{L-lactate} + O_2 + \text{LOD} \rightarrow \text{Pyruvate} + H_2O_2$$

This method of $[La^-]_B$ analysis has been validated against a classical spectrophotometric method (Sigma) which yielded the following correlation:

$$y = 0.99x - 0.05 \ (\text{mmol.L}^{-1}) \ r = 0.996$$

where:

N = 24
x = spectrophotometric method
y = Analox

A single point calibration was performed prior to blood sampling. An adapted positive displacement pipette (Analox) injected 7µl of a known calibration standard (8 mmol.L^{-1} of $[La^-]_B$ into the analyser cuvette. The interpolation of lactate concentration using this standard has been identified to be linear up to a concentration of 10 mmol.L^{-1}. This was deemed sufficient as a pilot study demonstrated that the protocols employed in this investigation rarely exceeded 8 mmol.L^{-1}. A quality control material (Analox lactate/pyruvate quality control serum) was used to confirm reagent activity and to correct calibration.

3.6.6 Measurement of Serum Urea

Activation of the purine nucleotide cycle or catabolic wasting of skeletal muscle yields ammonia which is extremely toxic and is converted to non-toxic urea for urinary excretion A comprehensive overview of the biochemical pathways involved in the "Krebs-Henseleit ornithine cycle" is presented in Salway (1995, p40-41).

Concentrations of serum urea were assessed using a portable reflectance photometer (Refletron®). Prior to the analysis of a blood sample, the performance of the optical system was checked with Check strips (Refletron®) that measure the amount of light that has been diffusely reflected from the strip at 3 different wavelengths (567 nm, 642 nm and 951 nm)

expressed in $^{0}/_{00}$. Quality control material (Precinorm U) was also used to ensure analytical accuracy prior to blood sampling.

Arterialised capillary blood (32 µl) was collected in a heparanised capillary tube and subsequently injected onto a urea strip using a positive displacement pipette (Refletron®). A reagent carrier separates erythrocytes and plasma reacts with urease (≥5.3 U) and a buffer (34 µg). Urea is hydrolysed to form ammonium carbonate which releases ammonia in the presence of an alkaline buffer. The ammonia subsequently diffuses through a hydrophobic layer and causes a partial change in the buffer indicator layer. The colour of the indicator changes from yellow to green and then to blue. The concentration of the modified indicator is proportional to the urea concentration and the reflectance measured at 642 nm (Refletron® Manual). This method has been validated against the classical enzymatic UV method which yielded the following regression equation:

$$y = 1.188 + 0.00x \text{ (mmol.L}^{-1}\text{)} \quad r = 0.998$$

where:

N = 100

x = Reference method (enzymatic UV test)

y = Refletron® Urea method

3.7 HYDRATION STATUS

3.7.1 Measurement of Urine Volume

An estimation of daily fluid balance was made by measuring total fluid intake and total fluid loss (total urine volume + sweat loss) during a 2 day period. Urine samples were collected into plastic containers which were subsequently weighed to calculate urine volume (assuming 1kg = 1L). Exercise sweat rates (ml.min^{-1}) were calculated by measuring nude body mass immediately pre and post exercise.

3.7.2 Measurement of Urine Osmolality

Urine osmolality was determined using an Advanced Instruments Incorporated™ Micro-Osmometer, Model 3MO plus (Massachusetts, USA). The principle of operation is based

on a supercooling procedure and the subsequent measurement of the freezing point of a sample.

Prior to urinary analysis, the instrument was calibrated with 2 or more calibration standards of known concentrations (NaCL ± 2 mOsm-kg H_2O) at each of 2 calibration levels. A sampler (Advanced Instruments Incorporated™ Micro-Osmometer, Model 3MO plus) was used to inject 20µl of a mid-flow urine sample into the micro-osmometer. Sampling repeatibility is reported to be ± 2 mOsm-kg H_2O (0-400 mOsm-kg H_2O) and ± 0.5% at 400 - 2,000 mOsm-kg H_2O (Manual).

3.8 CARDIOVASCULAR MEASUREMENTS

3.8.1 Laboratory Heart Rate

Each subject was prepared for bipolar 3 lead electrocardiography. The skin surface was gently abrased using cotton wool and cleaned with swabs saturated with 70% v/v Isopropyl alcohol (Medi Swab, Smith and Nephew, UK). To ensure quality readouts by minimising resistance across the electrodes, body hair was removed in male subjects using a razor (Universal, UK). Three Ag/AgCl electrodes (Skintact^R) were applied to the following anatomical sites; Right arm (below right clavicle, midway between sternum and shoulder), Left arm (below left clavicle, midway between sternum and shoulder) and V5 (at the horizontal level of V4 at left anterior axillary line). Transpore™ surgical tape (3M, USA) was used to secure the electrodes and trailing wires in place. Heart rate was displayed continuously at rest and during exercise using a Rigel 304 heart rate monitor (Graseby Medical Limited, UK). The ECG monitor was interfaced to an on-line computerised gas analysis system and printed on-line at 30s intervals. A screen was erected to obscure the heart rate monitor from the subject's view during the course of all laboratory tests. The average HR during the last 60 s of either submaximal or maximal exercise was used during data analysis.

3.8.2 Track Heart Rate

An ECG calibrated short-range telemetry system (Polar Vantage NV™, Polar Electro Oy, Finland) was used to monitor HR during a standardised track session. HR's were sampled and averaged every 5 s and transmitted via electromagnetic waveforms to a watch receiver. Each subject depressed a button on the watch to "mark" both the beginning and the end of

each repetition which was automatically stored in a file. The watches were downloaded at the end of the track session using a Polar Advantage Interface™ which was connected to a lap top computer (Toshiba, T1910, 486 SX). A software package (Polar®, version 5.03 for Microsoft Windows) was used to analyse HR data. The average HR during the final 30 s of each repetition and the average recovery HR recorded 30 s immediately after completion of each repetition was used in the overall analysis.

3.8.3 Orthostatic Tolerance Test (Δ Heart Rate)

Each subject performed a modified orthostatic tolerance test which was originally developed by Czajkowski (1982) as a simple means of evaluating the physiological implications of intensive endurance training in elite cross country skiers. Heart rates were determined immediately on waking in a supine position and following 20 s of standing using Polar Vantage NV™ heart rate monitors or by individual palpation of the radial artery. The difference was calculated as delta heart rate (Δ HR).

3.8.4 Validation of Heart Rate Measurement

A pulse meter, (Rigel ECG Stimulator, 202, Graseby Medical Limited, UK) was connected to the Rigel ECG monitor and Polar Vantage heart rate monitor to determine the accuracy of heart rate measurement. One investigator set the pulse meter to a known frequency and a second "blinded" investigator recorded the average heart rate measurements in beats per minute ($b.min^{-1}$) during a 60 s period. This procedure was repeated in an randomised fashion for a number of heart rate frequencies ranging from 50 $b.min^{-1}$ to 200 $b.min^{-1}$. A one-way analysis of variance for independent samples identified that there were no differences in HR between monitors. (Appendix G).

3.8.5 Arterial Oxygen Saturation (SaO_2)

Resting arterial oxygen saturation (SaO_2) was displayed continuously throughout the test with an ear oximeter (Biox 3000, Ohmeda). The oximeter shines red and infrared light through the tissue and detects the fluctuating signals caused by arterial blood pulses. The ratio of the fluctuation of the two colour signals received determines the SaO_2:

$$SaO_2 = f \quad \frac{\text{In (min/max)}_{R}}{\text{In (min/max)}_{IR}}$$

where:
f - function
$_{R/IR}$ - Red light measured at 660 nm/Infrared light measured at 910 nm

The accuracy of this procedure has been documented as ± 2% (at an SaO_2 of between 70 - 100%).

3.8.6 Blood Pressure

Systemic arterial blood pressure (BP-mmHg) was measured in the brachial artery non-invasively using the auscultatory method outlined by the American Society of Hypertension Public Policy Position Paper (1992). Using a mercury sphygmomanometer (Accoson *Freestyle*, Cardiokinetics, UK) and stethoscope (Littmann, 3M, USA), systolic pressure was recorded at the first appearance of clear repetitive tapping sounds (Korotkoff Phase 1) and diastolic pressure was recorded at the disappearance of repetitive sounds (Korotkoff Phase 5). In some subjects in whom sounds continued until the zero point, diastolic pressure was determined at the distinct muffling of repetitive sounds (Korotkoff Phase 4).

3.8.7 Perceptual Scales

Each subject subjectively rated how hard they were working during exercise using the original Borg scale ratings of perceived exertion (Borg, 1973). This scale ranges from 6 to 20 (Appendix H). Subjects were instructed to indicate their perception of exercise intensity by pointing at the scale.

3.9 RESPIRATORY MEASUREMENTS

3.9.1 Measurement of Oxygen Uptake ($\dot{V}O_2$)

Experimental logistics necessitated the use of two automated on-line gas analysis systems and one manual reference system to measure ventilatory and pulmonary gas exchange parameters during exercise. The systems used were [1] Jaeger EOS-Sprint (Market Harborough, UK), [2] MedGraphics^R Cardiopulmonary exercise systems CPX/D, Cardiokinetics, UK) and [3] Douglas Bag method.

3.9.1.1 Jaeger EOS-Sprint

Subjects breathed through a broad flanged rubber mouthpiece that was connected to a low resistance (< 5cm H_2O at 300 $L.min^{-1}$), low dead space (<50 ml) Salford two-way non-

rebreathing respiratory valve. Expired airflow was directed to a pneumotacograph (Jaeger PT 18/20) via 1.5 m of Falconia tubing (Internal diameter - 4 cm). The pneumotacograph was maintained at 40°C via a thermal feedback device and manually calibrated at a variety of flow rates with a 3 L syringe. Expired air was dried using copper sulphate crystals and presented to a mixing bag where gas was analysed at a rate of 0.5 $l.min^{-1}$ by fast-response paramagnetic oxygen (O_2) and infrared carbon dioxide (CO_2) analysers. The gas analysers were calibrated with ambient air and a high quality calibration gas mixture which contained 14.88% O_2, 4.94% CO_2 and balanced N_2 (BOC Special Gases). Oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) respiratory exchange ratio (RER) and expired minute ventilation ($\dot{V}_E$) were expressed at standard temperature and pressure dry (STPD at 0°C, 760 mmHg and 0mmHg saturated water vapour pressure). All respiratory data were sampled at 30 sec intervals and printed on-line (Epson LX800, UK).

3.9.1.2 MedGraphics CPX/D

The MedGraphics CPX/D on-line gas analysis system utilises a breath-by-breath measurement technique. This analyser incorporates a pneumotach system which consists of a "preVent" pneumotach (valve dead space = 20 ml) and transducer. This was calibrated at 5 different flow rates using a 3 L syringe to verify a linear response prior to experimentation. Signals were directed to a waveform analyser which converts the analog signals to correlate with flow. A computer (Mitsibushi, Japan, 286) integrated flow relative to time to obtain a volume measurement. Dried expired gas was presented to fast-responding infra red CO_2 and zirconium O_2 analysers which were calibrated using a high quality reference gas (21% O_2, balanced N_2) and a calibration gas [(5% CO_2, 12%O_2 and balanced N_2), BOC Special Gases]. Respiratory parameters expressed at STPD were sampled every 30 s and printed on-line (Citizen Swift 200, UK).

3.9.1.3 Douglas Bag Method

Each subject breathed through a rubber mouthpiece connected to a low resistance, low dead space (<50 ml) Salford breathing valve (Jakeman and Davies, 1979). Expired air was directed via Falconia tubing (1.5 m at 32 mm ID) into a series of Douglas Bags (150L) via two way stop valves. The collection of expired gas was timed using a chronograph (Timex) to the nearest whole breath for a 60 s period at standardised time intervals. The total resistance to flow was 2.9 cm H_2O at a constant flow of 200 L.min-1 which conformed with

the World Health Organisation (WHO) recommendations (Andersen et al, 1971). The volume of expired gas was measured with a dry gas meter (Harvard Limited, Edenbridge, UK) which had been validated against a 600 L Tissot spirometer (Collins Limited, USA). Aliqouts of expired gas (2.0L) were dried using fresh copper sulphate crystals and presented to paramagnetic O_2 (Servomex 570A, Crowborough, UK) and infra-red CO_2 (Servomex PA 404, Crowborough, UK) analysers for the determination of percentage O_2 and CO_2. The analysers had been calibrated using precision-analysed quality gas mixtures (BOC Special Gases). Computation of oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) corrected to STPD was computed using the Haldane Transformation (Wasserman et al, 1994).

3.9.1.4 Validation of Respiratory Measurements

Both the Jaeger EOS-Sprint and MedGraphics CPX/D on-line analysers were validated by comparing respiratory data measured during a standardised $\dot{V}O_{2max}$ test with an off-line reference system. Details of the procedures involved are discussed in Appendix I.

3.9.2 Measurement of Pulmonary Function

Pulmonary function was measured using flow loop spirometry (Cosmed Kit, Cosmed SRL, Rome, Italy). Each subject assumed an upright seated position and breathed into a disposable cardboard mouthpiece (22 mm diameter) connected to a bi-directional turbine flow meter with a dynamic resistance of <1.5 cm H_2O $L.sec^{-1}$ at a flow rate of 12 $L.sec^{-1}$ Pulmonary data was analysed using a portable lap top computer (Toshiba T1910, 486 SX). Following a maximal inspiration, each subject performed a maximal expiration which they were instructed to hold for 6 s for the determination of forced vital capacity (FVC), forced expiratory volume in 1 sec (FEV_1), mid-expiratory flow rates ($FEF_{25-75\%}$), peak inspiratory flow rates (PIF) and peak expiratory flow rates (PEF). All indices of pulmonary function were expressed as a % or in $L.min^{-1}$ (BTPS). Each test was repeated until the computer software indicated that reproducible flow loops had been obtained. Following a 2 minute recovery, each subject was instructed to maximise tidal volume and breathing frequency during a 12 sec period for the determination of maximal voluntary ventilation (MVV) expressed in $L.min^{-1}$ (BTPS). Individual values were compared to "normal" values based on regression equations developed by Ceca (1971, 1983).

3.10 STATISTICAL METHODS

3.10.1 Power of the Test

Prior to studies 1 and 2, the mean sample size required for the detection of a statistically significant difference ($P < 0.05$) as a function of a treatment effect (hypobaric hypoxia) was determined for a variety of dependent variables according to the procedure of Altman (1980). This procedure required the determination of the critical difference (Fraser and Fogarty, 1989) for a variety of cardiorespiratory and metabolic parameters. A discussion of this procedure is outlined in Appendix J.

3.10.2 Descriptive Statistics

Data were analysed using a computerised statistical package (SPSS for MS Windows Release 6.1) using both parametric and non-parametric statistics where applicable and descriptive values are reported as means ± 1SD. Non-parametric statistics were incorporated if there was evidence of marked heterogeneity of variance or if the data were skewed. This was ascertained using the Shapiro-Wilk W test for Normality (Altman, 1991) and the Normal P-P Plot (Kinnear and Gray, 1994). The latter method plots the cumulative proportion for a single numeric variable against the cumulative proportion expected if the sample were from a normal distribution. If the sample is from a normal distribution, points will cluster around a straight line as illustrated in Figure 3.1 below:

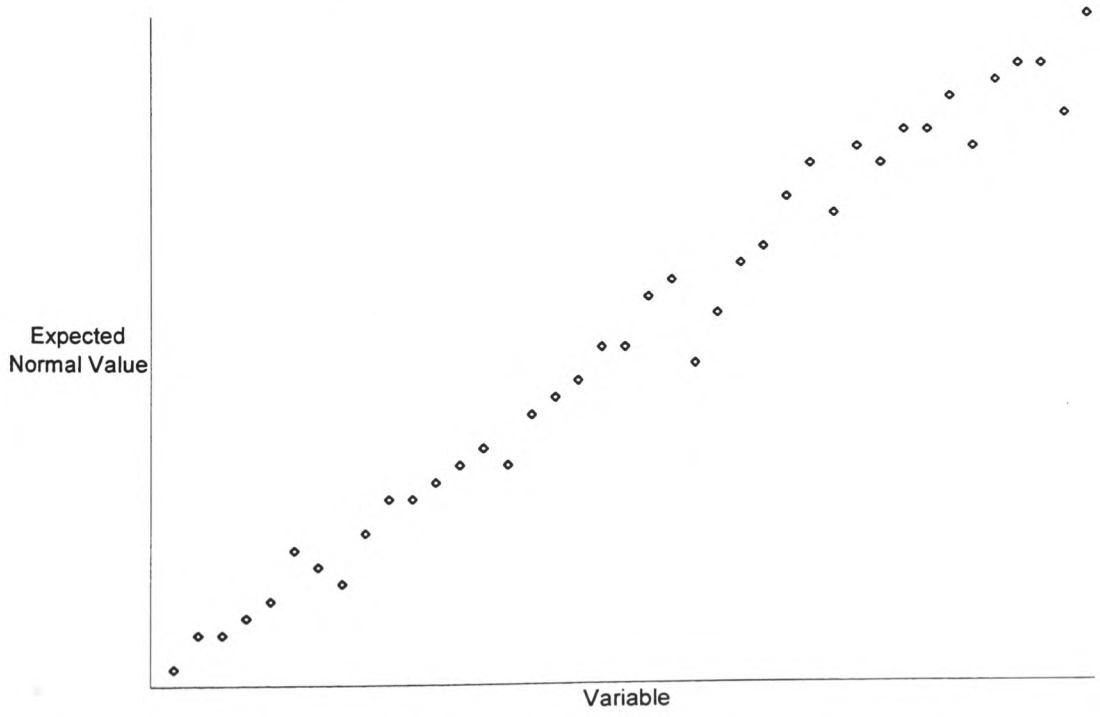


Figure 3.1 Normal P-P Plot Demonstrating a "Normal Distribution"

3.10.3 Within Group Comparisons

After verification of a normal distribution (Gaussian graphical verification), a one factor repeated measures analysis of variance (ANOVA) was conducted to determine differences between EXP group mean values (PRE vs ALT vs POST). This statistic incorporates the Mauchly Sphericity Test to evaluate the homogeneity of covariance assumption which is important for the univariate approach (Howell, 1992 and Kinnear and Gray, 1994). If this test was significant ($P < 0.05$), a more conservative test (Greenhouse-Geisser) was used (Howell, 1992; Kinnear and Gray, 1994). When the ANOVA F-ratio was significant, a *posteriori* Tukey's Honestly Significant Difference test (Tukey, 1977) was employed to locate significant differences. The Friedman test (Friedman, 1937) served as the non-parametric equivalent.

A paired samples *t-test* was used to compare changes in dependent variables between PRE and POST tests. The Wilcoxon Matched-Pairs Signed-Ranks test (Kinnear and Gray, 1994) was employed as the non-parametric equivalent.

The relationship between two dependent variables was ascertained parametrically using the Pearson Product Moment Correlation test (Kinnear and Gray, 1994) or the Spearman Rank Correlation (r_s) as the non-parametric equivalent (Altman, 1991) and graphically represented by fitting individual data points with either a linear or exponential regression line on a scatterplot.

3.10.4 Between Group Comparisons

A t-test for independent samples was conducted to compare means taken from the two groups of subjects (EXP and CON). This test incorporated the Levene test (Kinnear and Gray, 1994) to determine homogeneity of variance. The Mann-Whitney U test (Mann and Whitney, 1947) served as the non-parametric equivalent.

Statistical significance was defined at the $P < 0.05$ level for all two-tailed tests.

3.11 EXPERIMENTAL OVERVIEW

The physiological implications of moderate altitude training (1,500-2,000 m) on endurance performance at altitude and following return to sea-level were determined in two separate investigations that were conducted over a twelve month period. A detailed discussion of these investigations will be discussed in Chapters 5 and 6.

Study [1] New Mexico Altitude Training Camp (USA)
Determination of the effects of 4 weeks of moderate altitude training (1,500-2,000 m) on physiological indices of submaximal and supramaximal running performance at altitude and following return to sea-level.

Study [2] Krugersdorp Altitude Training Camp (S.Africa)
Determination of the effects of 4 weeks of moderate altitude training (1,640 m) on physiological indices of maximal and supramaximal running performance at altitude and following return to sea-level.

The inclusion of a field test, undertaken on a tartan track was considered to be an important characteristic of this study and one which has been ignored by the vast majority of the scientific literature related to altitude training. Several hypoxic training studies have also failed to incorporate a normoxically trained control group in their experimental design (Bailey and Davies, 1997) which makes it impossible to determine whether the physiological changes that occur following a bout of altitude training can be attributed to an improvement in physical conditioning or to the additive effects of hypoxia *per se* (Section 2.7). Therefore, particular attention focused on incorporating a performance matched control group who lived and trained at sea-level, whilst experimental subjects attended the respective altitude training camps.

CHAPTER 4

STUDY 1: NEW MEXICO

THE EFFECTS OF MODERATE ALTITUDE TRAINING ON PHYSIOLOGICAL INDICES OF SUBMAXIMAL AND SUPRAMAXIMAL PERFORMANCE

4.1 INTRODUCTION

It is clear from the data presented in Tables 2.10 and 2.11 that the majority of scientific research has investigated the effects of altitude training on $\dot{V}O_{2max}$, whilst few studies have documented changes in physiological indices of *submaximal* exercise performance. Gleser and Vogel (1973) expressed the relationship between a subject's endurance time at a given exercise intensity and $\dot{V}O_{2max}$:

$$\log (t) = A \cdot (\dot{V}O_{2max}/\text{load}) + B$$

where:

t - endurance time

A - constant related to the rate of anaerobic metabolism

B - constant related to the quantity of anaerobic metabolism

This equation clearly demonstrates that a change in $\dot{V}O_{2max}$ is not necessary for an improvement in endurance performance. Over the last decade, an accumulating body of research has clearly demonstrated that cardiorespiratory and metabolic measures of *submaximal* exercise performance provide a more sensitive means of evaluating a treatment effect (Jakeman et al 1993 and Sjodin and Svedenhag, 1994). Thus, the purpose of the present study was to evaluate the effects of moderate altitude training on both *submaximal* (work output < $\dot{V}O_{2max}$) and *supramaximal* (work output > $\dot{V}O_{2max}$) indices of physiological performance.

4.2 METHODS

4.2.1 Selection of Subjects

Sixteen male and seven female subjects (n = 23) were recruited from a pool of International standard distance runners who were sea-level natives. Subject recruitment was conducted in conjunction with the National Endurance Coach for 5 km to 10 km events.

4.2.2 Subject Characteristics

All subjects were International standard middle to long distance runners, of whom two were Commonwealth Games medallists. Their anthropometric, dietary and track running performance data are summarised in Tables 4.1 and 4.2.

Table 4.1 Anthropometric and Dietary Characteristics of Subjects (n = 23)

Dependent Variable	Group Mean ± SD (Range)
Age (Years)	24 ± 4
	(18 - 32)
Body Mass (Kgs)	61.5 ± 7.5
	(45.4 - 73.2)
Stature (m)	1.74 ± 0.09
	(1.63 - 1.89)
Body Fat (%)	11.6 ± 5.5
	(5.1 - 24.8)
Systolic Blood Pressure (mmHg)	123 ± 12
	(100 - 150)
Diastolic Blood Pressure (mmHg)	77 ± 10
	(60 - 100)
Forced Vital Capacity (L).	4.85 ± 0.88
	(3.34 - 6.22)
Daily Calorific Intake (KCal)	2832 ± 525
	(1841 - 3809)
Daily Carbohydrate Intake (g.kg bwt^{-1})	6.5 ± 1.8
	(4.4 - 10.7)
Daily Carbohydrate Intake	52 ± 6
(% of total calorific intake)	(44 - 64)

Table 4.2 Track Running Performance Data (n = 23)

Distance (m)	♂ n	♀ n	♂ Group Mean (Range)	♀ Group Mean (Range)
800*	4	1	1:50 (1:48 - 1:54)	2:02
1,500*	4	0	3:45 (3:41 - 3:51)	.....
3,000	1	4	8:28	9:30 (9:05 - 9:55)
5,000	6	2	13:47 (13:10 - 14:02)	15:47 (15:10 - 16:24)
10,000	2	0	28:42 (28:29 - 28:54)	.....

Values are expressed in minutes : seconds

*: some subjects are classified as 800 and 1,500 m specialists (both times are included in data analysis)

The subjects were subsequently separated into two groups that were matched for running performance. Ten males and four female subjects (n = 14) were assigned to an altitude training camp which was to be based at Albuquerque, New Mexico, USA (EXP). The remaining six males and 3 females subjects (n = 9) continued with their normal training programme at sea-level in the UK (CON). EXP and CON group anthropometric and activity data are summarised in Table 4.3.

Table 4.3 Group Anthropometric and Activity Data

Dependent Variable	EXP	CON
Age (Years)	24 ± 4	25 ± 4
Body Mass (Kgs)	62.0 ± 8.7	60.9 ± 6.0
Stature (m)	1.75 ± 0.11	1.73 ± 0.06
Body Fat (%)	11.9 ± 6.0	11.2 ± 5.3
Forced Vital Capacity (L)	4.81 ± 0.99	4.90 ± 0.77
Running Distance ($km.week^{-1}$)	74 ± 24	68 ± 16

Values are Mean ± SD.
EXP: Altitude group (n = 14)
CON: Sea-level group (n = 9)

4.2.3 Experimental Design and Protocol

A matched two group cross-sectional design was adopted during study 1 (Figure 4.1). An elite male 800/1,500 m specialist volunteered for a pilot study prior to study 1 to assess the practicality of the experimental procedures involved.

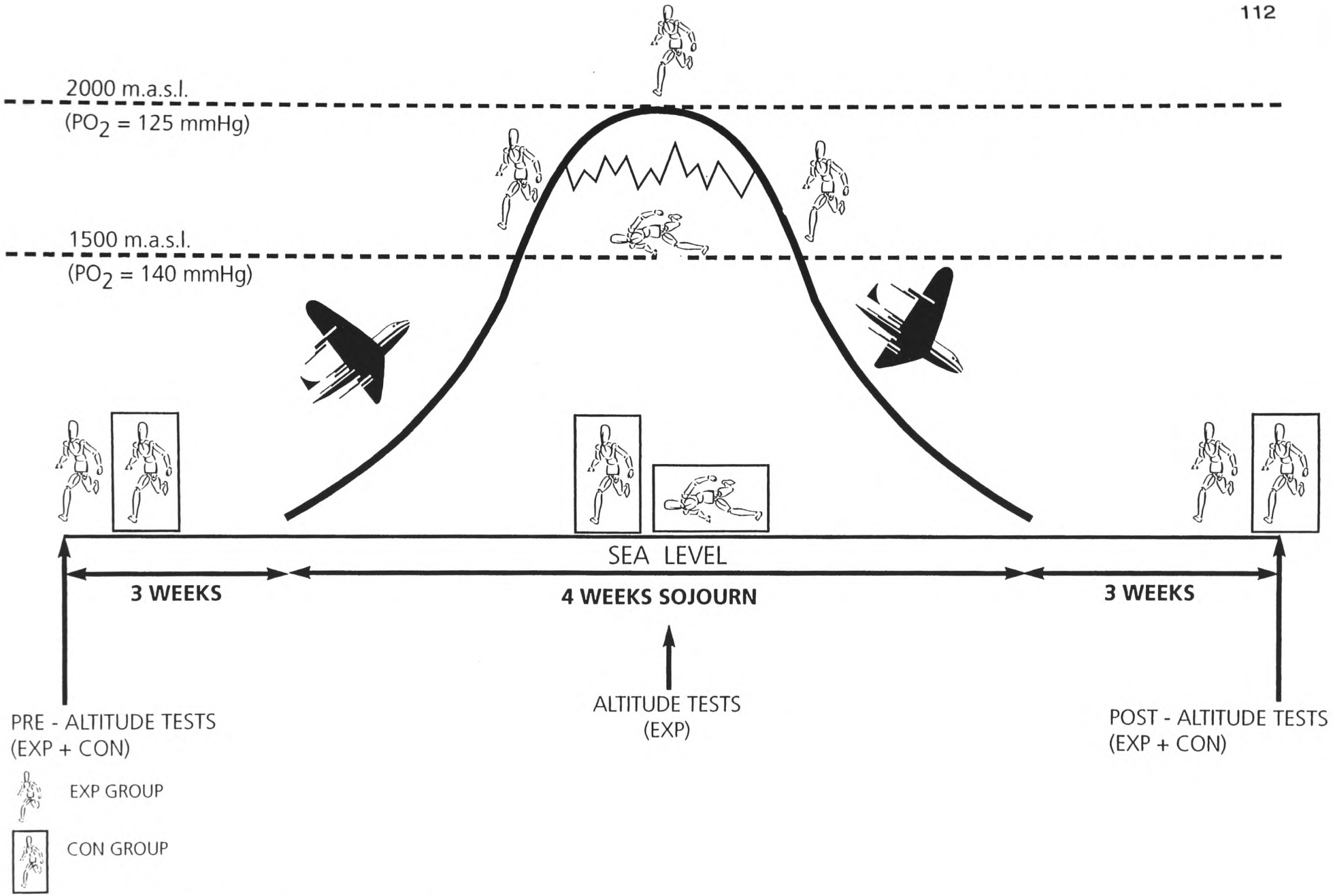


Figure 4.1 Experimental Design (New Mexico)

4.2.3.1 Pre-Altitude Testing (PRE)

All pre-altitude physiological tests were conducted at sea-level approximately 3 weeks prior to the start of an altitude training camp which was held at Albuquerque, New Mexico, USA (~1,500-2,000 m). Each subject in the EXP group was provided with an altitude training guideline booklet which contained information on immunisation, an altitude acclimatisation strategy and how to recognise and prevent the symptoms of dehydration.

4.2.3.1.1 Resting Measurements

Each subject reported to the laboratory at least 3 h post prandial during which resting anthropometric, metabolic and cardiovascular data were collected. A detailed description of the analytical procedures involved are discussed in Chapter 3.

4.2.3.1.2 Laboratory Test

A minimum of two treadmill sessions were performed prior to study 1 to control for the confounding effects of habituation (Appendix K). Data collected during a pilot study were used to design a suitable submaximal treadmill test which is outlined below:

A diagrammatic representation of the laboratory protocol is illustrated in Figure 4.2. Following a 4 minute warm-up at 2.78 $m.s^{-1}$ and 5 minutes of flexibility and calisthenics, each subject performed a discontinuous incremental treadmill run which consisted of 5 incremental stages each of 4 minutes in duration. The treadmill velocities were calculated to represent from between 70 to 110% of the subject's individual 10 k personal best running velocity on the road (Treadmill velocity = 3.56 to 5.86 $m.s^{-1}$ for male subjects and 3.19 to 5.06 $m.s^{-1}$ for female subjects).

Respiratory gas exchange parameters ($\dot{V}_E$, $\dot{V}O_2$, $\dot{V}_E/\dot{V}O_2$ and RER) were measured during the last 60 s of each treadmill stage with an open-circuit system which has been described in detail in the previous chapter. HR was determined via three lead bipolar electrocardiography and displayed continuously throughout the test. Subject RPE was determined during the last 10 s of each increment. The subject straddled the moving treadmill belt for 30 s at the end of each treadmill stage for the collection of an earlobe blood sample which was subsequently analysed for $[La^-]_B$. A 10 minute warm down at 2.78 $m.s^{-1}$ marked the end of the treadmill test which lasted a total of 41 minutes.

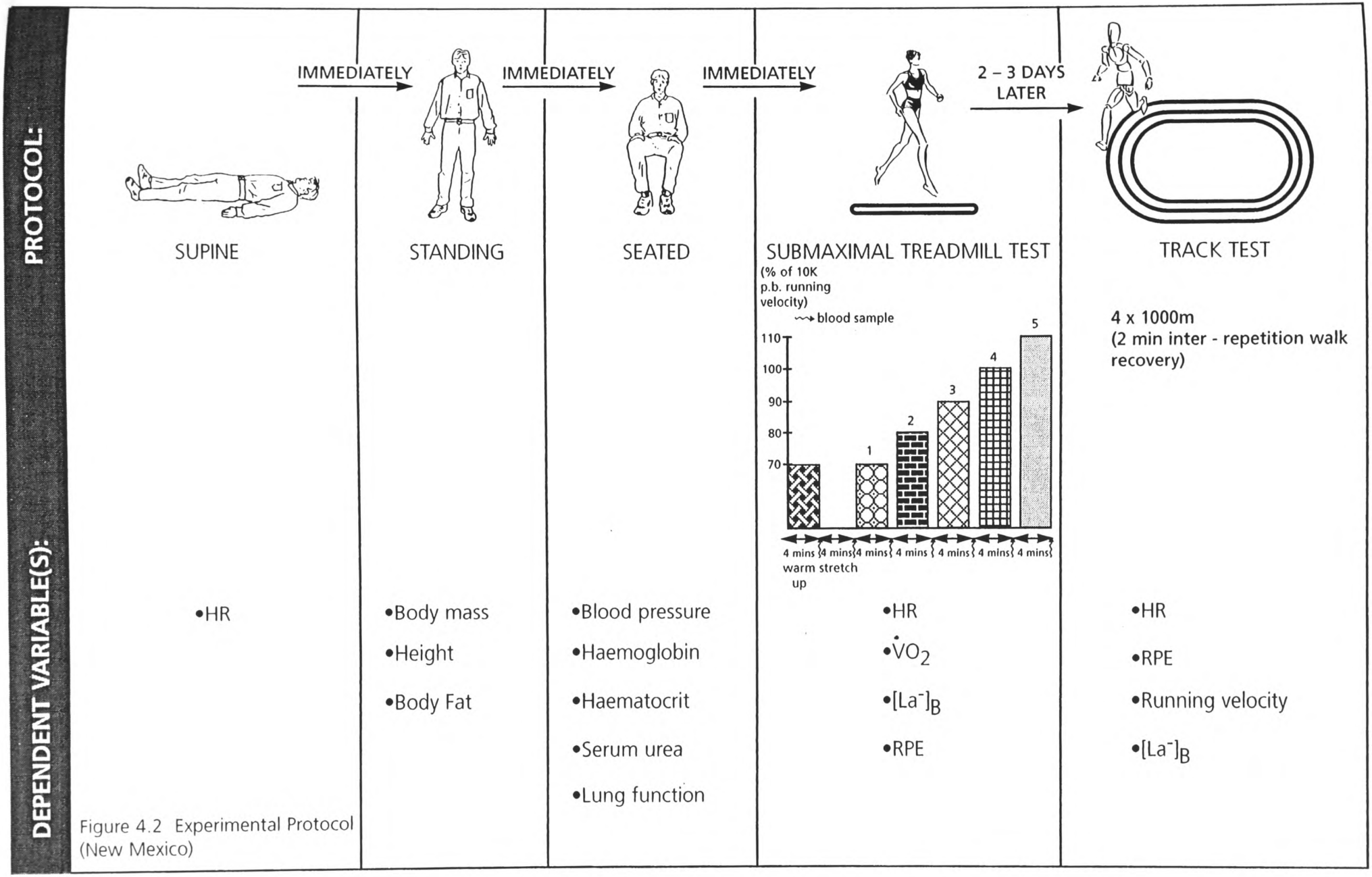


Figure 4.2 Experimental Protocol (New Mexico)

4.2.3.1.2.1 Determination of the Lactate Threshold: θ $[La^-]_B$

Since its conception in 1964 by Wasserman and McIllroy, the "anaerobic threshold" has been the source of much controversy amongst exercise physiologists. Defined as "the level of work or O_2 consumption just below that at which metabolic acidosis and the associated changes in gas exchange occur" (Wasserman et al 1973), there is still much debate regarding physiological mechanisms, interpretation, precise detection and acceptable nomenclature (Loat and Rhodes, 1993). However, both the ventilatory and metabolic thresholds are sensitive methods used to evaluate endurance capacity or to assess the effect of training (McLellan and Cheung, 1992).

The θ $[La^-]_B$ in the present study was determined using the Dmax method which has been shown to be an objective and reliable method for threshold determination (Cheng et al 1992). A diagrammatic representation of this procedure is illustrated in Figure 4.3. A third order polynomial regression was fitted to a scatterplot of $\dot{V}O_2$ (x axis) against $[La^-]_B$ (y axis). A straight line was fitted to the two end points of the curve (A and B) and the maximal distance (Dmax) between the line and the curve was calculated mathematically. The interpolated $\dot{V}O_2$ was taken as the threshold.

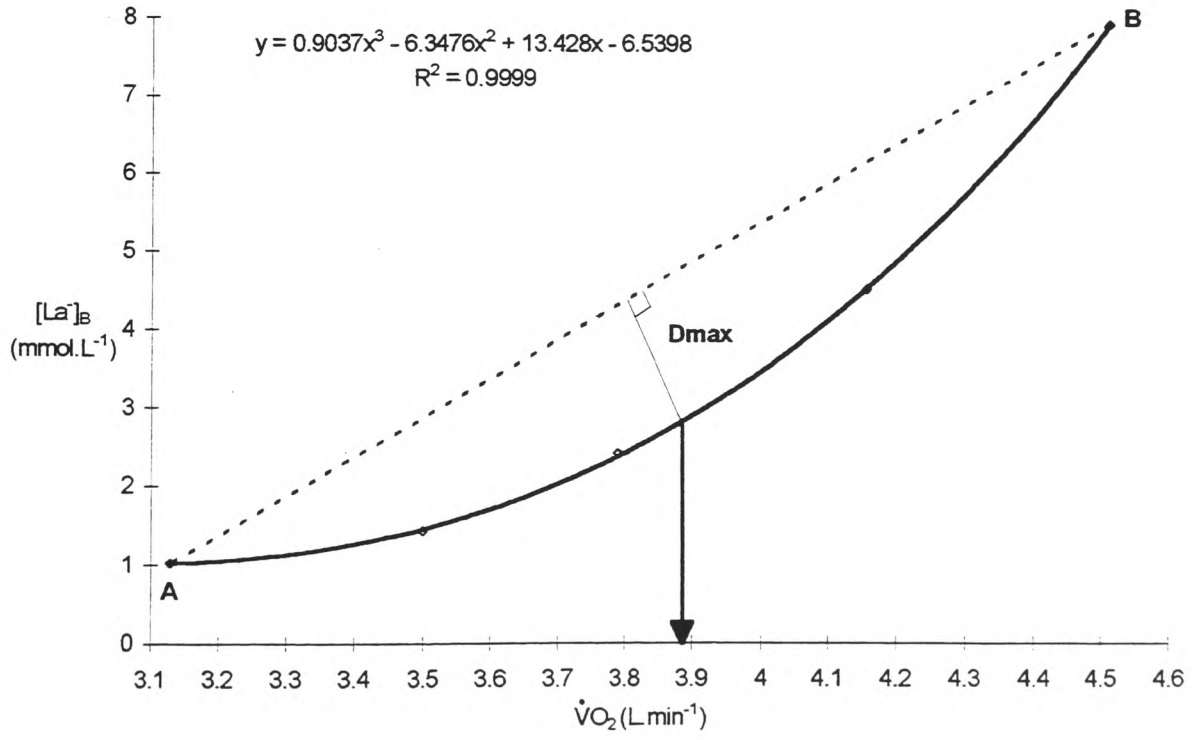


Figure 4.3 Determination of θ $[La^-]_B$ According to the Dmax Method (Cheng et al 1992)

4.2.3.1.3 Track Test

Each subject performed a standardised track session at sea-level two to three days following the laboratory test. The track session consisted of four repetitions of 1000 m on a tartan track, separated by a 2 minute recovery walk. The average HR during the final 30 s of each repetition and the average recovery HR recorded 30 s immediately after completion of each repetition was recorded using bipolar telemetry. Subject RPE was determined immediately on completion of each repetition and an earlobe blood sample was collected and subsequently analysed for $[La^-]_B$.

Each repetition was performed at a velocity that equated to the subject's 3 km personal best time to ensure that all 4 repetitions were performed at a consistent velocity. Running velocity was controlled by the National Endurance Coach (5 km to 10 km) who recorded each 400 m split time and informed the subject who subsequently adjusted their running velocity accordingly.

4.2.3.1.4 Training Load

Each subject recorded total weekly running distances and corresponding heart rates one week prior to PRE and POST testing. A blood sample was collected immediately following a typical steady state run lasting for 45 to 60 minutes for the determination of $[La^-]_B$. Training distances were divided into track (HR~170-185 $b.min^{-1}$) and steady state (HR~140-160 $b.min^{-1}$) running sessions. All subjects were instructed to continue with their current training programme throughout the duration of the study.

4.2.3.2 Altitude Testing (ALT)

4.2.3.2.1 Resting Measurements

All physiological and medical equipment was flown to Albuquerque, USA and implemented into a portable laboratory based at 1,500 m. Physiological testing was organised in collaboration with Dr R.Robergs (Director of the Human Performance Laboratory, University of New Mexico, Albuquerque, USA). Resting HR, blood pressure, Hb, Hct, serum urea and body mass were determined on a daily basis between 0600 h and 1100 h during days 8 to 19 at altitude. These measurements were performed on each subject immediately on waking and following an overnight fast.

Daily fluid balance was estimated by subtracting total fluid loss (urine output + sweat loss) from total fluid intake over a 48 h period. Sweat loss was determined by measuring nude body mass pre and immediately following a standard road run. It was assumed that any decrease in body mass could be attributed to a loss of fluid among the plasma, extracellular and intracellular space (Maughan, 1994).

4.2.3.2.2 Track Test

A track session was repeated on day 16 on a tartan track based at 1,500 m. Each subject was instructed to perform the session as consistently as possible (similar repetition velocities) under the guidance of the National Endurance Coach.

4.2.3.2.3 Training Load

The EXP group was instructed to perform all altitude training sessions at the same relative exercise intensity as the training conducted previously at sea-level (i.e. same heart rate). Each subject recorded weekly running distance and intensity during the 4 weeks at altitude.

An attempt was made to quantify the physiological implications of training at altitude during a typical steady state run that lasted between 45 to 60 minutes. An earlobe blood sample was collected immediately post exercise from six male and 2 female subjects (n = 8) and subsequently analysed for $[La^-]_B$. HR was determined using short-range bipolar telemetry.

A portable altimeter was fitted to a male subject (5,000 m specialist) for the duration of the altitude sojourn to determine the ambient PO_2's and corresponding altitudes attained during individual training sessions.

4.2.3.3 Post-Altitude Testing (POST)

All tests performed during pre-altitude testing were repeated 3 weeks following the EXP group's return to sea-level.

Experimental data were analysed using a variety of parametric and non-parametric ranking statistical tests which are discussed in detail in Chapter 3.

4.3 RESULTS AND DISCUSSION

4.3.1 Environmental Conditions

The unpredictability of environmental conditions at altitude makes it difficult to isolate the effects of hypoxia *per se* on physiological function. The confounding influences of changing temperature and relative humidity were encountered in the present study and are summarised in Figures 4.4 and 4.5.

Barometric pressure averaged 758 mmHg at sea-level (Range: 756 mmHg - 758 mmHg) and decreased to a mean of 631 mmHg at altitude (Range: 628 mmHg - 637 mmHg). The ambient temperature at sea-level averaged 9.1°C (Range: 4.3°C - 13.8°C) which rose to a mean value of 14.7°C at altitude (Range-1.9°C - 22.8°C). Snow had fallen on the 15th day at altitude which made it particularly difficult for the subjects to train. Relative humidity decreased from a mean value of 43% at sea-level (Range: 41% - 45%) to 38% during the altitude sojourn (Range: 25 - 66%).

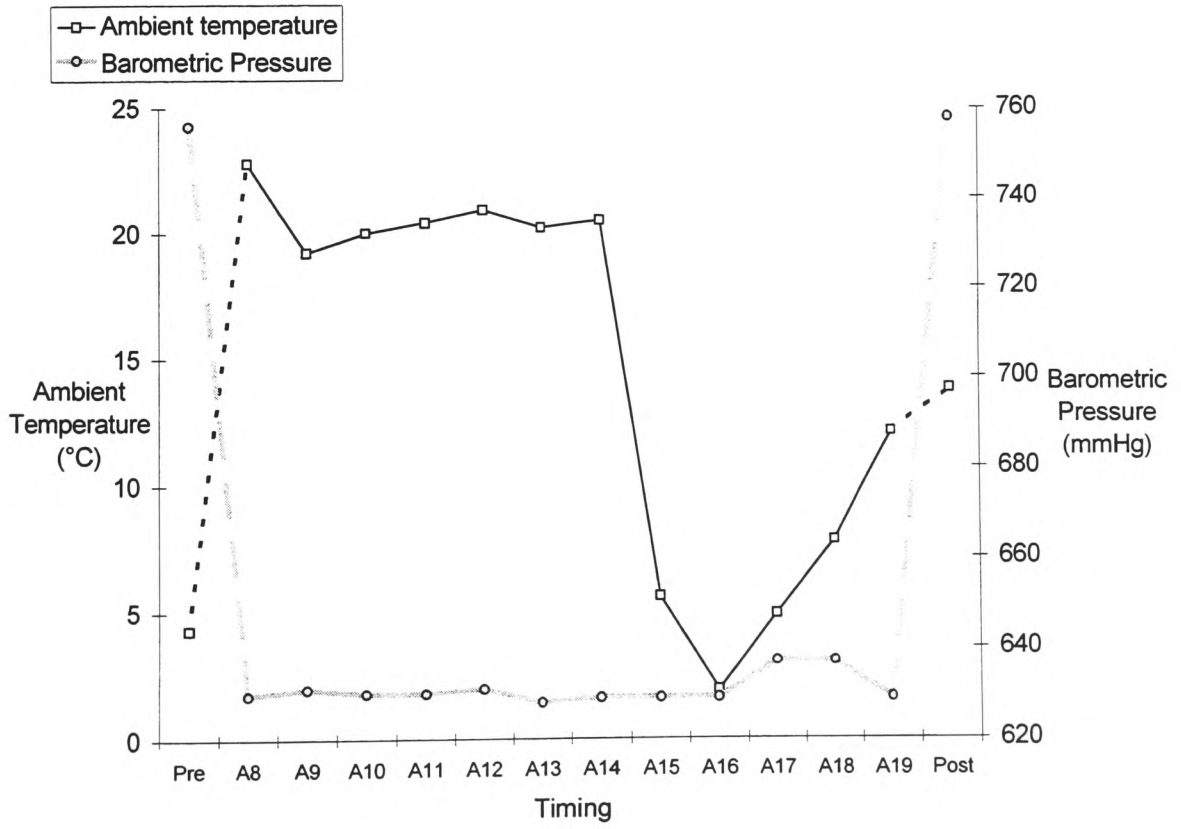


Figure 4.4 Ambient Temperature and Barometric Pressure at Sea-Level and Altitude
Pre: Pre-altitude
A8 - A19: Days 8 to 19 at altitude (1500 m)
Post: Post-altitude

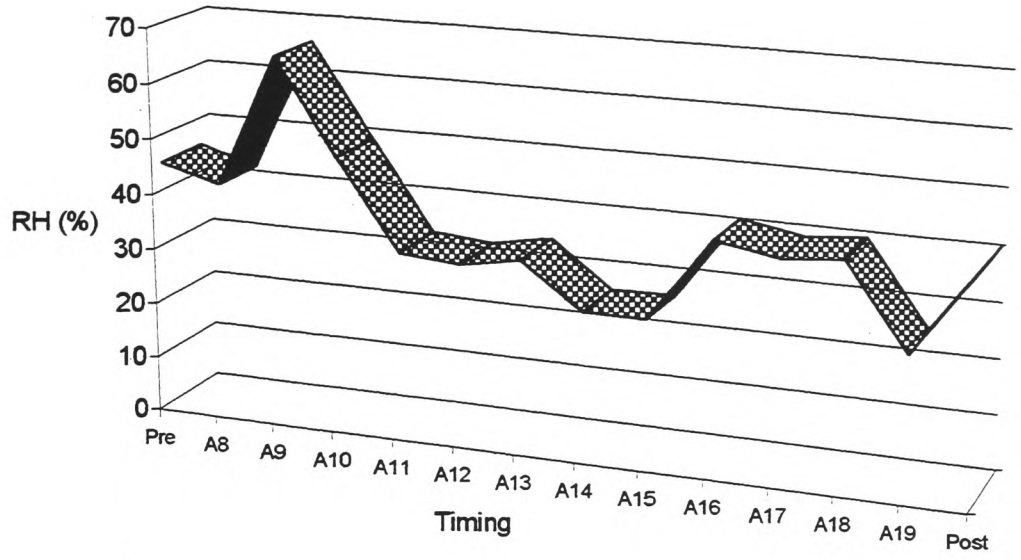


Figure 4.5 Relative Humidity at Sea-Level and Altitude
Pre: Pre-altitude
A8 - A19: Days 8 to 19 at altitude (1500 m)
Post: Post-altitude

4.3.2 Training Load

Quantification of training load at altitude is an important parameter which has been considered by few studies which makes them difficult to interpret due to the training effects that may occur independently of hypoxia.

Figure 4.6 and Table 4.4 summarise weekly training volume and intensity during the experimental period. There were no significant differences between PRE EXP and PRE CON group mean steady state (HR~ 140-160 $b.min^{-1}$), track (HR~ 170-185 $b.min^{-1}$) or total weekly running distances. EXP group mean weekly steady state and total running distance was significantly lower ($P < 0.01$ vs PRE) during weeks 3 and 4 at altitude due to illness or injury. EXP track distances remained stable throughout. There were no changes in CON group steady state, track or total weekly running distances during the study.

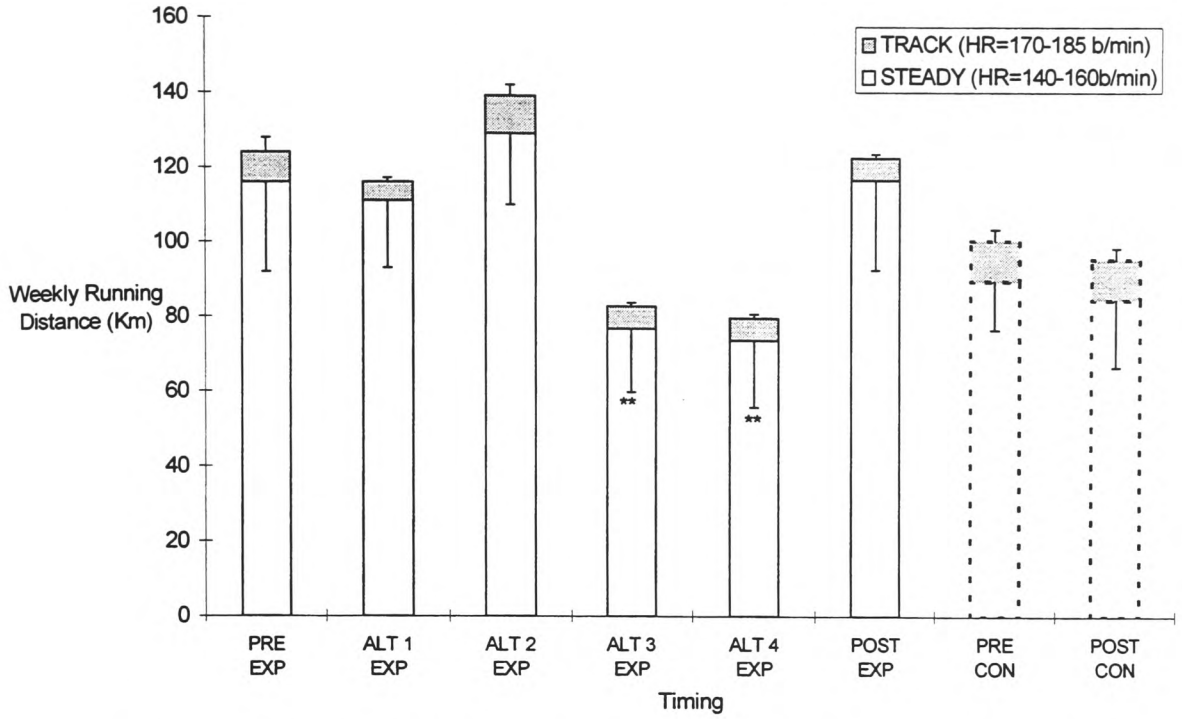


Figure 4.6 Weekly Track and Steady State Running Distances
Values are Mean ± SD
**: Significantly different from PRE value ($P < 0.01$)
PRE: Pre-altitude
ALT1-4: Weeks 1 to 4 at altitude (1500 m)
POST: Post-altitude

Table 4.4 Weekly Running Distance at Sea-Level and Altitude

Timing	Group	Weekly Distance (km)	Range (km)
PRE	EXP	124 ± 40	53 - 177
ALT (WEEK 1)	EXP	116 ± 29	74 - 169
ALT (WEEK 2)	EXP	137 ± 34	77 - 177
ALT (WEEK 3)	EXP	82 ± 27‡	42 - 130
ALT (WEEK 4)	EXP	81 ± 31‡	42 - 130
POST	EXP	122 ± 40	64 - 177
PRE	CON	103 ± 23	72 - 145
POST	CON	98 ± 29	48 - 145

Values are Mean ± SD (Range)
‡: Significantly different from PRE value ($P < 0.01$)
PRE: Pre-altitude
ALT: Altitude
POST: Post-altitude
EXP: Altitude group (n = 9)
CON: Sea-level group (n = 9)

A general overview of the type of training sessions conducted on consecutive days during physiological testing at altitude is illustrated in Table 4.5. These sessions were conducted at a variety of different altitudes ranging from between 1,500 to 2,000 m (Figure 4.7).

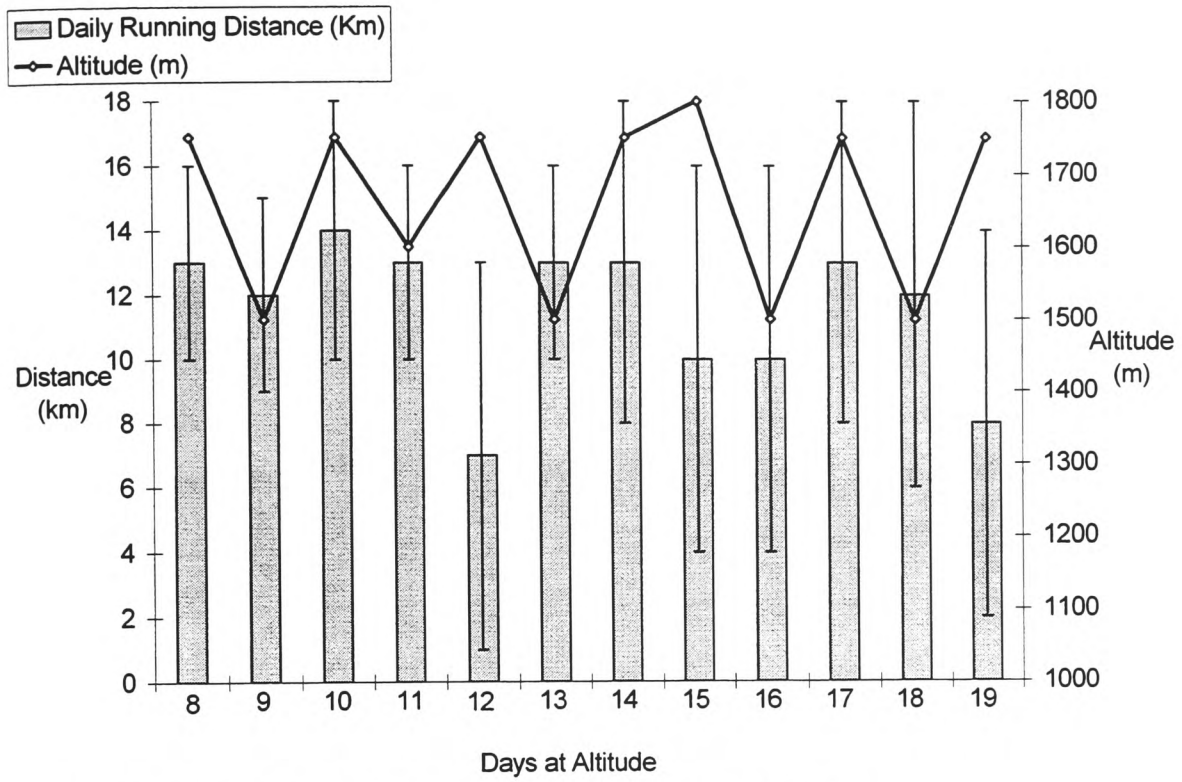


Figure 4.7 Typical Training Profile During the Altitude Sojourn
Values are Mean ± SD

Table 4.5 Training Sessions Conducted at Altitude (~1,500-2,000 m)

Day at Altitude	Session (pm)*
8	Steady road run
9	Track session (400 m repetitions with a 90 s inter-rep recovery)
10	Steady road run
11	Maximal steady state road run
12	Steady road run
13	Hill sprints (15-25 s efforts) with a walk-back recovery
14	Steady road run
15	Steady road run
16	Track session (1,000 m repetitions with a 120 s inter-rep recovery)
17	Steady road run
18	Steady road run
19	Steady road run

*: All pm sessions were preceded by an early morning 6-8 km easy road run (HR~140-160 $b.min^{-1}$)

The data presented in Table 4.6 would suggest that the relative work rate at altitude was comparable to that encountered at sea-level. However, whilst running velocity for a given training HR at altitude was not determined in the present study, it was likely that the mountainous terrain would have caused a decrease in absolute training intensity. Stine et al (1992) demonstrated that steady exercise was performed at a lower running velocity even at 1,200 m in a group of competitive distance runners. This has been demonstrated to induce a detraining response and thus mask any potentially favorable physiological adaptations invoked during acclimatisation (Levine et al, 1996). Thus, it is possible that the subjects in the present study may have experienced some degree of detraining whilst training at altitude.

Table 4.6 Post Exercise Heart Rate (HR) and Whole Blood Lactate Concentration ($[La^-]_B$) at Sea-Level and Altitude (1,500 - 2,000 m)

Dependent Variable	PRE EXP	ALT* EXP
HR ($b.min^{-1}$)	140 ± 12	142 ± 8
Range ($b.min^{-1}$)	122 - 158	123 - 167
$[La^-]_B$ ($mmol/L^{-1}$)	2.70 ± 0.90	2.83 ± 0.86
Range ($mmol.L^{-1}$)	2.35 - 3.05	2.00 - 3.9

Values are Mean ± SD and Range.
*: Measurements made during week 3 at altitude.
PRE: Pre-altitude
ALT: Altitude
EXP: Altitude group (n = 9)
CON: Sea-level group (n = 9)

4.3.3 Resting Adaptations

A summary of the changes in selected physiological parameters measured at rest during the course of experimentation is illustrated in Tables 4.7 to 4.12.

4.3.3.1 Anthropometric Adaptations

Primary anorexia, lack of palatable food, detraining and the direct effects of hypoxia *per se* on protein synthesis have been implicated in the weight loss that has been documented at altitude (Kayser, 1994). However, it is unlikely that weight loss below 5,000 m would occur assuming adequate dietary intake. This was borne out in the present study (Table 4.7) and would suggest that daily energy expenditure approximated daily energy intake. Whilst a dietary analysis was not conducted at altitude, subjects from the EXP group were advised to increase their carbohydrate intake for a number of reasons; although equivocal, an accelerated glycolytic flux has been demonstrated in response to environmental hypoxia which has been associated with increased circulating adrenaline concentrations (Mazzeo et al 1989 and Young et al 1991). Whilst a decrease in muscle glycogen concentration increases the activity of glycogen synthase in normoxia (Adolfsson and Ahren, 1971 and Piehl et al 1974), there is evidence to suggest that hypoxia *per se* may depress glycogen resynthesis (Milledge et al 1976), possibly due to a decrease in testosterone which is implicated in the conversion of inactive glycogen synthase into its active form (Adolfsson, 1971).

Table 4.7 Anthropometric Measurements at Sea-Level and Altitude (1,500 m)

Variable	PRE EXP	ALT* EXP	POST EXP	PRE CON	POST CON
Body Mass (Kgs)	62.4 ± 9.2	62.5 ± 9.5	62.5 ± 9.2	60.9 ± 6.0	60.2 ± 5.9
Range (Kgs)	45.4 - 73.2	46.1 - 74.8	46.8 - 74.8	49.7 - 66.7	49.3 - 66.0
Body Fat (%)	12.8 ± 6.7	.....	11.7 ± 7.3	11.2 ± 5.3	11.2 ± 5.0
Range (%)	5.1 - 24.8	.....	5.1 - 24.8	5.5 - 19.4	5.2 - 19.0

Values are Mean ± SD
*: ALT values represent mean values obtained between days 8 - 19 at 1,500 m.
PRE: Pre-altitude
ALT: Altitude
POST: Post-altitude
EXP: Altitude group (n = 9)
CON: Sea-level group (n = 9)

4.3.3.2 Resting Haematological Adaptations

4.3.3.2.1 Hydration Status

Water metabolism at altitude is controversial and possibly reflects the technical and invasive nature of the measurement of total body water content (Young and Young, 1988). However, it is clear that acute exposure to environmental hypoxia causes a disruption in fluid homeostasis possibly due to an uncoupling of the control mechanism which regulates the renin-angiotensin-aldosterone system, (Fulco and Cymerman, 1988) and the release of atrial natriuretic peptide (Lawrence and Shenker, 1991). Increased albumin catabolism and decreased synthesis may also be implicated in the fluid losses noted at altitude (Surks, 1966). Although these systems tend to stabilise during chronic exposure to environmental hypoxia, a decrease in intra and extracellular water is accompanied by a decrease in plasma volume during the early stages of altitude acclimatisation (Milledge, 1992b and Kayser, 1994). However, there are few studies that have investigated fluid loss or redistribution at moderate altitudes such as that encountered in the present study.

Whilst it is possible that a decrease in plasma volume may have resulted in a corresponding haemoconcentration, the data presented in Table 4.8 would suggest that the contaminating effects of dehydration at altitude were minimal. Between one to two years prior to this study, nine out of the 10 subjects had previously attended a warm weather training camp. Each subject in the EXP group had also received a dehydration-rehydration briefing at sea-

level prior to the altitude sojourn and was thus considered experienced in recognising and preventing the physiological symptoms associated with dehydration. The EXP group appeared to be in a euhydrated state as noted by a marginally positive fluid balance (+0.02 ± 2.50 L) and it is likely that the contribution of metabolic water at altitude which has been estimated at 1L.day^{-1} for metabolic work rates equivalent to 11.7 - 15.6 MJ.day^{-1} (Westerterp et al 1992) would further improve hydration status.

Table 4.8 24 Hour Fluid Balance at Altitude (1,500 m, n = 10)

Variable	X ± SD	Range
Urination Frequency	10 ± 3	7 - 17
Urine Voided (L)	2.25 ± 1.32	0.81 - 4.57
Steady Run Sweat Rate (ml.min^{-1})*	16.5 ± 6.1	9.7 - 29.2
Total Run Fluid Loss (L)*	1.48 ± 0.55	0.87 - 2.63
Resting Fluid Loss (L)#	1.42 ± 0.33	1.00 - 2.00
Total Fluid Loss (L)	5.15 ± 1.49	3.32 - 8.07
Drinking Frequency	8 ± 2	4 - 12
Fluid Intake (L)	5.17 ± 2.96	2.48 - 10.14
Fluid Balance (L)^	+0.02 ± 2.50	-2.74 - [+5.89]

Values are Mean ± SD and Range
Calculations represent mean values determined over a 2 d period during week 3 at altitude
*: Calculated during a steady state road run (90 minutes at HR~140-160 b.min^{-1}) at an ambient temperature of 5.6°C and 66% relative humidity
#: Determined during a rest day at an ambient temperature of 5.6°C and 66% relative humidity (n = 5)
^: Excludes the metabolic production of water

4.3.3.2.2 Haemoglobin (Hb) and Packed Cell Volume (PCV)

Table 4.9 demonstrates that in comparison to PRE EXP group mean values, EXP group mean resting Hb and PCV concentrations did not change significantly at altitude (ALT EXP) or following return to sea-level (POST EXP).

Table 4.9 Resting Haemoglobin (Hb) and Packed Cell Volume (PCV) at Sea-Level and Altitude (1,500 m)

Variable	PRE EXP	ALT* EXP	POST EXP	PRE CON	POST CON
Hb (g/dl)	14.5 ± 1.7	15.3 ± 1.2	15.1 ± 1.5	15.2 ± 1.3	15.1 ± 1.3
Range (g/dl)	12.4 - 17.6	13.1 - 17.2	11.6 - 16.7	13.9 - 17.2	12.8 - 16.4
PCV (L/L)	0.44 ± 0.04	0.45 ± 0.03	0.45 ± 0.05	0.47 ± 0.03	0.46 ± 0.03
Range (L/L)	0.36 - 0.48	0.39 - 0.49	0.37 - 0.52	0.43 - 0.49	0.39 - 0.48

Values are Mean ± SD.
*: ALT values represent mean values obtained between days 8 - 19 at 1,500 m.
PRE: Pre-altitude
ALT: Altitude
POST: Post-altitude
EXP: Altitude group (n = 9)
CON: Sea-level group (n = 9)

However, the haematological response on consecutive days at altitude was highly variable (Figures 4.8 and 4.9) and it is difficult to resolve the physiological mechanisms responsible for the significantly elevated Hb concentrations on days 14, 16, 17 and 18 ($P < 0.05$ vs PRE EXP) and PCV on days 16 at altitude ($P < 0.01$ vs PRE EXP). A "true" increase in Hb may have occurred due to an elevated reticulocytosis mediated by increased concentrations of erythropoietin (EPO) at altitude. Whilst EPO was not determined in the present study, an investigation by Roberts and Smith (1992) has demonstrated a 21% increase in resting EPO ($P < 0.05$ vs pre-altitude) and a corresponding 7% increase in resting Hb concentration ($P < 0.05$ vs pre-altitude) in world ranked swimmers who trained at 1,850 m.

Alternatively, a haemoconcentration due to a loss of total body water and/or redistribution of plasma water to extravascular spaces may have artificially elevated resting Hb/PCV concentrations. In comparison to a mean ALT EXP Hb concentration of 15.3 ± 1.2 g/dl and PCV of 0.45 ± 0.03 L/L, the changes in Hb and PCV on days 14, 16, 17 and 18 at altitude would equate to a 9% decrease in resting plasma volume following application of the Dill and Costill equation (1974).

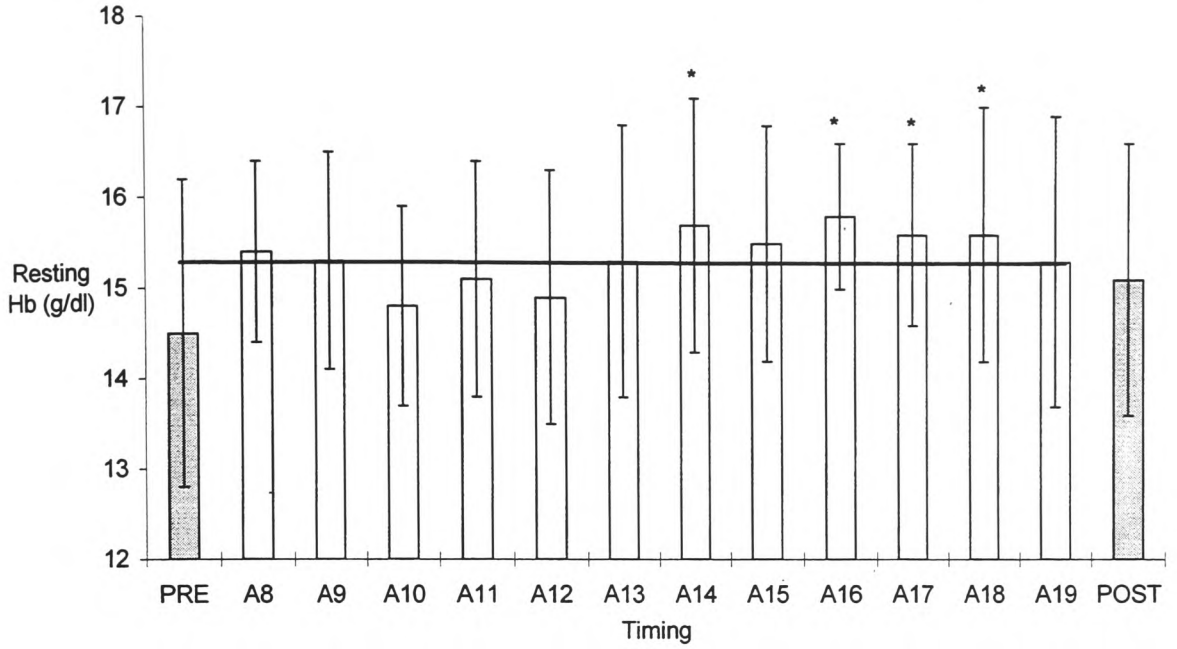


Figure 4.8 EXP Group Resting Hb Concentration at Sea-Level and Altitude
Values are Mean ± SD
*: Significantly different from PRE value ($P < 0.05$)
PRE: Pre-altitude
A8 - A19: Days 8 to 19 at altitude (1500 m)
POST: Post-altitude
Emboldened line represents mean value during the altitude sojourn

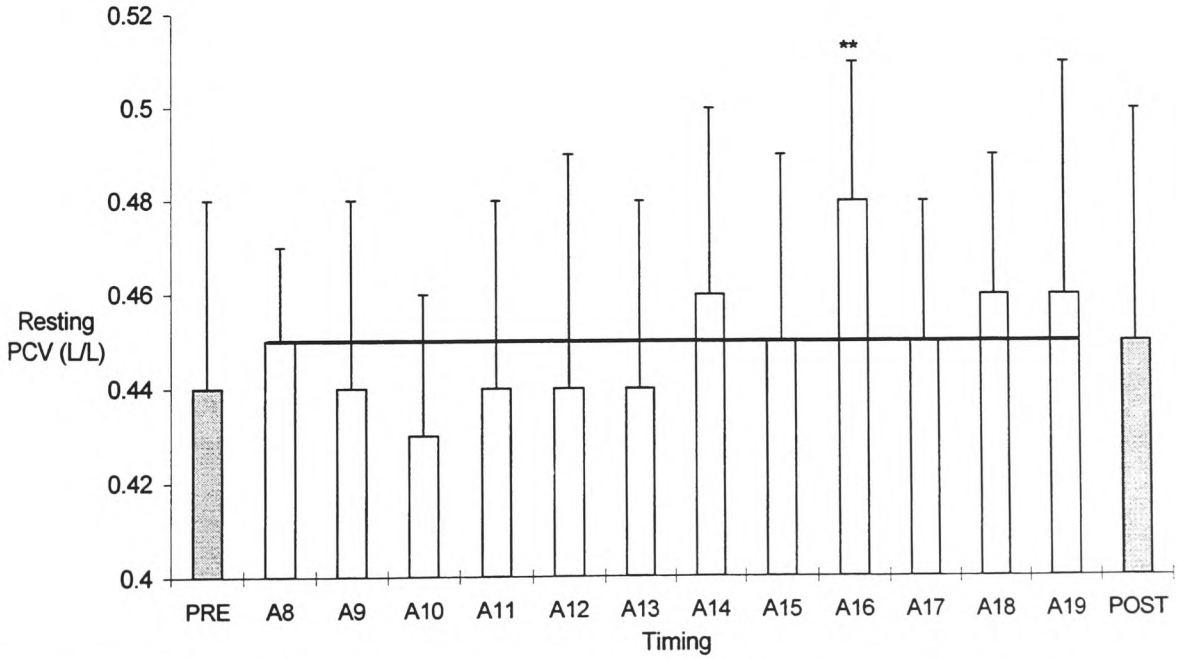


Figure 4.9 EXP Group Resting PCV Concentration at Sea-Level and Altitude
Values are Mean ± SD
**: Significantly different from PRE value ($P < 0.01$)
PRE: Pre-altitude
A8 - A19: Days 8 to 19 at altitude (1500 m)
POST: Post-altitude
Emboldened line represents mean value during the altitude sojourn

Much attention has focused on maximising the rate of haematological adaptation at altitude which is sensitive to a variety of factors including [1] iron status (Stray-Gundersen et al 1992) [2] intensity and duration of the hypoxic stimulus (Weil et al 1968), and [3] time of descent to sea-level (Ingjer and Myhre, 1992). Berglund (1992) has reviewed the kinetics of the "erythropulse" at altitude and has summarised that in healthy subjects, resting Hb concentrations increase at a rate of 1% per week at moderate altitudes ranging from between 1,829 m to 3,048 m. EXP group mean resting Hb concentration on the last day of physiological testing at altitude in the present study ([ALT 19] -15.3 ± 1.6 g/dl) was 6% greater ($P < 0.05$) than the pre-altitude value (14.5 ± 1.7 g/dl), a rate of increase that was approximately double that predicted by Berglund (1994). The formation of EPO is governed by an intrarenal "oxygen sensor" that is modulated by extrarenal humoral or neural influences (Bauer and Kurtz, 1989). It is therefore possible that a more pronounced alveolar to arterial ($[A\text{-}a]O_2$) difference during exercise in the more aerobically trained athlete at altitude (Section 2.6.1.) could potentiate the hypoxic stimulus and thus account for an increased rate of haematological adaptation.

A negative correlation was observed ($r^2 = 0.58$, $P < 0.05$) between initial Hb concentration and the delta increase (ALT EXP mean - PRE EXP mean value) in resting Hb concentration at altitude (Figure 4.10). Thus, subjects with the lowest initial resting concentrations of Hb at sea-level experienced the largest increases during the altitude sojourn.

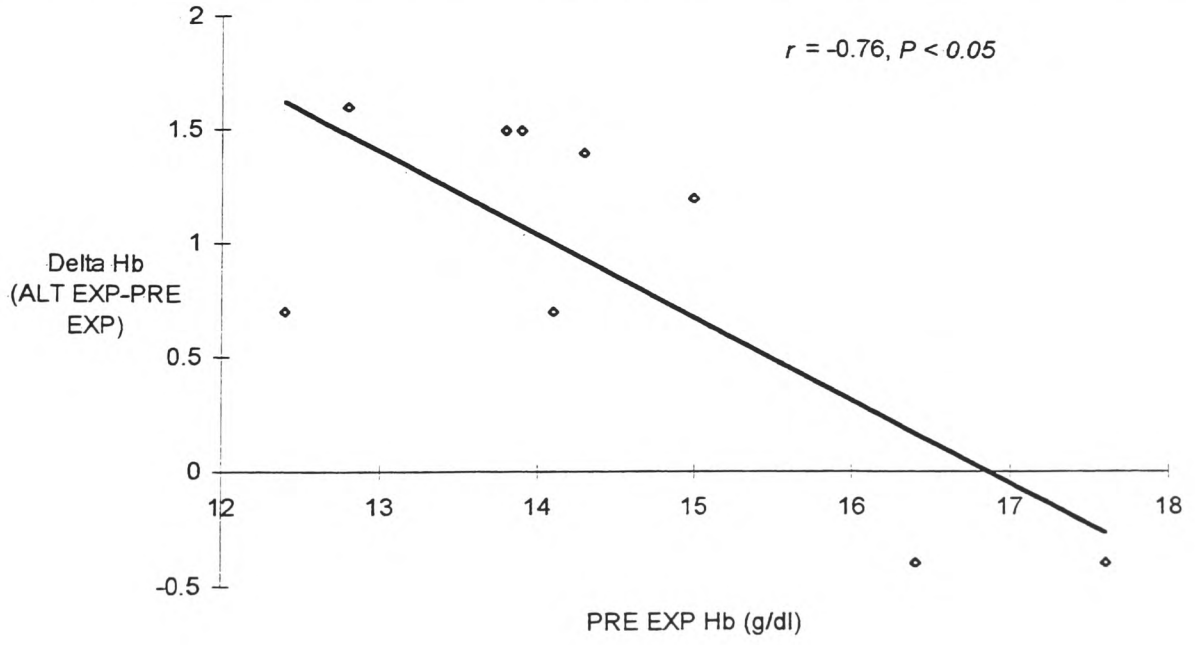


Figure 4.10 Changes in EXP Group Mean Haemoglobin (Hb) Concentration at Altitude

This observation was also noted by Ingjer and Myhre (1992) who studied the physiological implications of 3 weeks of altitude training at 1,900 m in a cohort of 7 elite male cross country skiers. The authors also demonstrated that those subjects who experienced the largest increases in resting Hb concentration at altitude also experienced the greatest decrease in $[La^-]_B$ during a standardised submaximal treadmill test 1 day following return to sea-level (r = 0.91). They attributed the reduced lactacidosis to an improvement in the oxygen transport/buffering capacity of the blood. However, there was no significant relationship between the increase in Hb concentration (POST EXP-PRE EXP) and the decrease in group mean $[La^-]_B$ during submaximal exercise in the present study (r = -0.70, P = 0.08). This would suggest that total oxygen delivery to the skeletal muscles, a product of arterial oxygen content (CaO_2) and flow probably remained unchanged. Table 4.10 demonstrates that estimated CaO_2 did not change appreciably as a function of the mean changes in Hb concentration. Resting arterial oxygen saturation (SaO_2) was not determined in the present study and values are derived from elite athletes based at a similar ambient PO_2 of 635 mmHg, equivalent to 1,520 m (Tucker et al 1984). Resting PaO_2 was derived from measurements made by Dempsey et al. (1984) using elite athletes exposed to a similar P_IO_2 (~130 mmHg).

Table 4.10 Estimation of Resting Arterial Oxygen Content at Sea-level and Altitude (n = 9)

Timing/ Group	Hb (g/dl)	SaO_2 (%)*	PaO_2 (mmHg)#	O_2 bound to Hb (mlO_2/dl)	Dissolved O_2 (ml/dl)	CaO_2 mlO_2/dl
PRE EXP	14.5	97.4	91	18.89 ± 2.15	0.27 ± 0.00	19.16 ± 2.15
ALT^ EXP	15.3	93	83	19.12 ± 1.46	0.25 ± 0.00‡	19.37 ± 1.46
POST EXP	15.1	97.4	91	19.71 ± 1.95	0.27 ± 0.00	19.98 ± 1.95
PRE CON	15.2	97.4	91	19.90 ± 1.67	0.27 ± 0.00	20.17 ± 1.67
POST CON	15.1	97.4	91	19.68 ± 1.66	0.27 ± 0.00	19.95 ± 1.66

Values are Mean ± SD

‡: Significantly different from within group PRE value (P < 0.01)

*: derived from Tucker et al (1984)

#: derived from Dempsey et al (1984)

PRE: Pre-altitude

^: Determined during days 19 to 20 at 1,640 m

$POST_2$: Determined 20 days following EXP group return to sea-level

EXP: Altitude group (n = 7)

CON: Sea-level group (n = 10)

A major criticism of the scientific studies that have investigated the effects of hypoxia-mediated secondary polycythaemia is their failure to account for changes in blood flow to skeletal muscle (Section 2.6.1). The poor relationship between increases in Hb following autologous blood reinfusion and $\dot{V}O_{2max}$ ($r = 0.52$) discussed in Section 2.4.2. suggests that an increase in Hb is not necessarily mirrored by an improvement in physical exercise capacity. The physiological responses to an elevated CaO_2 at altitude have been addressed in two studies (Boutellier et al 1982 and Wolfel et al 1991). Wolfel et al. (1991) investigated a variety of factors that could influence O_2 delivery at rest and during submaximal cycling exercise following acclimatisation to 4,300 m. They demonstrated that following 21 days of residence at altitude, resting and exercise cardiac output and iliac venous blood flow decreased significantly ($P < 0.05$ vs acute exposure and sea-level) due to increases in systemic and leg vascular resistance. Whilst CaO_2 increased after acclimatisation, the decrease in leg blood flow resulted in an unchanged O_2 delivery. The authors attributed the active vasoconstriction to a 28% increase ($P < 0.05$ vs pre-altitude) in circulating noradrenaline concentrations. They concluded that the regulation of O_2 transport during chronic hypoxia was related to reductions in central and peripheral blood flow, possibly caused by sympathetically mediated vasoconstriction. A decrease in muscle perfusion in response to an elevated CaO_2 has also been shown to persist following return to sea-level (Boutellier et al 1982).

4.3.3.2.3 Serum Urea Concentration

There were no differences observed in group mean resting serum urea concentration during the experimental period (Table 4.11).

Table 4.11 Changes in Resting Serum Urea Concentration at Sea-Level and Altitude (1,500 m)

Variable	PRE EXP	ALT* EXP	POST EXP	PRE CON	POST CON
Urea ($mmol/L^{-1}$)	5.63 ± 1.79	6.57 ± 1.26	6.01 ± 1.56	4.89 ± 1.26	5.52 ± 1.90
Range ($mmol.L^{-1}$)	3.33 - 8.24	4.56 - 8.24	3.92 - 8.78	3.33 - 7.60	3.33 - 9.7

Values are Mean ± SD and Range

*: ALT values represent mean values obtained between days 8 - 19 at 1,500 m

PRE: Pre-altitude

ALT: Altitude

POST: Post-altitude

EXP: Altitude group (n = 9)

CON: Sea-level group (n = 9)

There were no changes in the EXP group mean daily resting serum urea concentration on consecutive days at altitude (Figure 4.11).

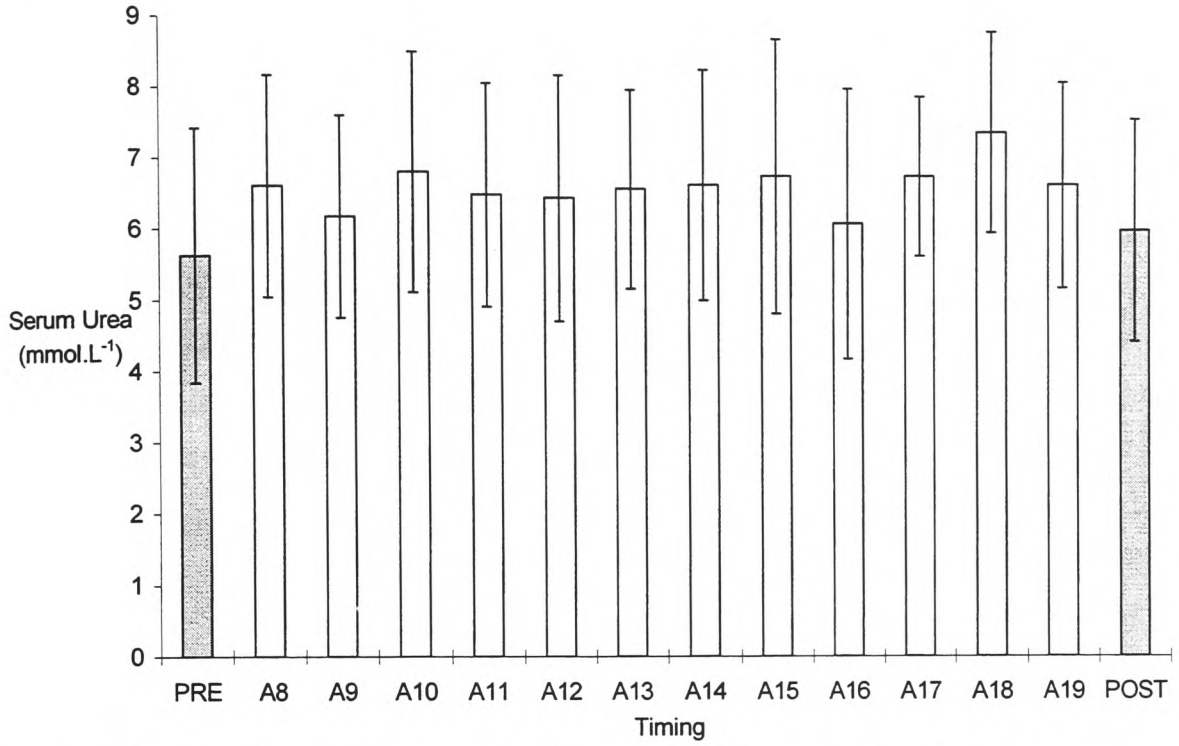


Figure 4.11 EXP Group Mean Resting Serum Urea Concentration (n = 9)
Values are Mean ± SD
No significant difference between PRE value ($P > 0.05$)
PRE: Pre-altitude
A8 - A19: Days 8 to 19 at altitude (1500 m)
POST: Post-altitude

Serum urea was incorporated as a metabolic indicator of physical stress in the present study. Identification of a reliable biochemical metabolite that is associated with overtraining has become the Holy Grail for exercise physiologists and biochemists alike for the last decade. There is some evidence to suggest that serum urea may represent such a marker. Activation of the purine nucleotide cycle (PNC) or amino acid deamination via the PNC or glutamate dehydrogenase are the major sources of toxic ammonium ions (NH_4+) in skeletal muscle (Terjung, 1996). NH_4+ is subsequently converted to non-toxic urea for urinary excretion by the Krebs-Henseleit ornithine cycle (Salway, 1995). Any NH_4+ which evades detoxification as urea is incorporated into glutamine by glutamine synthetase (Haussinger, 1989).

The evidence would suggest that increased resting or delayed recovery concentrations of NH_4+ are likely to contribute to the physical manifestations of "overtraining" (Sahlin, 1996), a complex syndrome which is characterised by underperformance and fatigue (Keast, 1988 and Kuipers and Keizer, 1988) . It has been suggested that the breakdown of adenine nucleotides to inosine monophosphate (IMP) and ammonia via the purine nucleotide cycle represents a condition of insufficient adenosine triphosphate (ATP) regeneration from adenosine diphosphate (ADP), consistent with muscle fatigue (Sahlin, 1996). An increase in plasma ammonia concentration has also been associated with disturbances in the central nervous system due to interference with neurotransmitter synthesis, in particular gamma amino butyric acid (Banister et al 1985), increased osmotic pressure and removal of α-ketoglutarate from the Krebs cycle (Stone et al 1991). Negative whole body nitrogen balance has also been reported in overtrained athletes (Noakes, 1986) and thus an increase in urea excretion derived from amino acid oxidation has been suggested as an index of overtraining (Janssen et al 1988). Amino acid oxidation, in particular the branched chain amino acids (BCAA) have been associated with increases in 5 hydroxytryptamine (5-HT) in the brain (Newsholme et al 1987) which is believed to contribute to central fatigue during prolonged exercise (Young, 1986). Parry-Billings et al. (1990) have also demonstrated that resting plasma glutamine concentration is significantly depressed ($P < 0.01$) in overtrained (chronically impaired exercise performance) as opposed to trained and untrained subjects. There is now emerging evidence which suggests that owing to the importance of glutamine to key cells of the immune system, in particular lymphocytes and macrophages (Ardawi and Newsholme, 1985), any decrease below a physiological concentration may cause immunosupression and underperformance (Rowbottom et al 1996). To this author's knowledge, the effects of environmental hypoxia on glutamine metabolism has not been investigated.

From the Geman work, Hollmann (1994) has suggested that resting serum urea concentrations should not increase above 8.5 $mmol.L^{-1}$ at altitude as this represents a catabolic situation that is associated with underperformance. As a consequence, some senior Olympic coaches train their athletes below this "urea threshold" to avoid overtraining (personal communication, J.Grobler, British Olympic Rowing Coach). Whilst group mean serum urea concentration did not change at altitude in the present study, several subjects exceeded the urea threshold on consecutive days at altitude (Figure 4.12).

However, there was no indication that these subjects were underperforming or suffering from extreme fatigue.

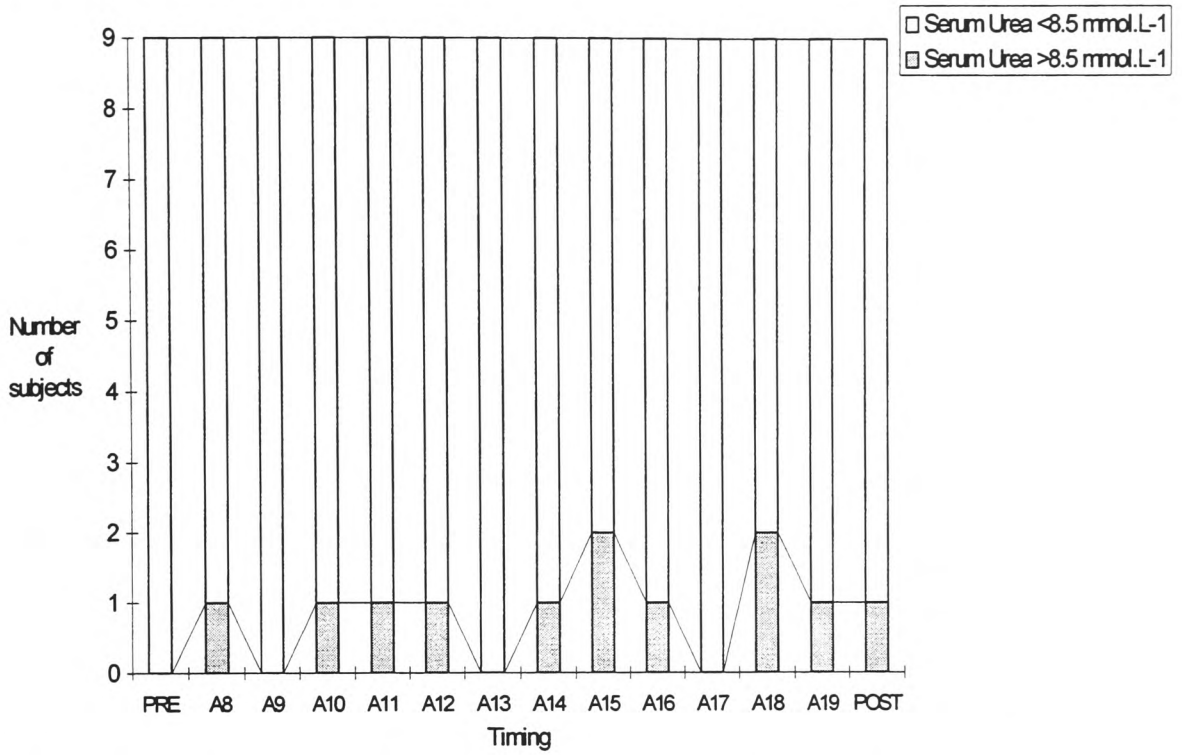


Figure 4.12 Frequency of EXP Subjects Above and Below the Urea Threshold (8.5 mmol.L^{-1})
Values are Means
PRE: Pre-altitude
A8 - A19: Days 8 to 19 at altitude (1500 m)
POST: Post-altitude

It should be noted that current interpretation of serum urea as a marker of overtraining or physiological stress is influenced by a number of factors. Firstly, unless urea concentration is determined using isotopic tracers such as [$^{15}N_2$], the recycling of urea via the urease reaction cannot be accurately quantified (Carraro et al 1993):

$$CO(NH_2)_2 + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

This metabolic pathway describes the diffusion of urea into the gut and its hydrolysis via the urease reaction to NH_3 which can then re-enter the Krebs-Henseleit ornithine cycle. Total urea production is also dependent on sex (Lemon, 1991) ambient temperature (Dolny and Lemon, 1988), muscle glycogen status (Lemon and Mullin, 1980), and a critical difference of 30% at the $P < 0.05$ level highlights the high biological variability of this metabolite (Fraser and Fogarty, 1989). Resting serum urea concentrations at altitude also

appeared to be influenced by event speciality (Bailey et al 1996). A trend towards higher EXP ALT serum urea was observed in male 800/1500 m subjects (n = 5) when compared to the male 5k/10k subjects (n = 5) at altitude (Figure 4.13); however, these differences attained significance on day 16 only at altitude ($P < 0.01$). It is likely that the 800/1,500 specialists have a higher percentage of Type II motor units which have been shown to contain higher activities of adenylate deaminase (Meyer and Terjung, 1979) which is the principle enzyme involved in the purine nucleotide cycle. It is equally possible that these subjects are more prone to glycogen depletion due to an increased reliance on glycogen as a substrate by the Type II muscle fibres (Thorstensson, 1976). Glycogen depletion has been demonstrated to either activate (Wagenmakers et al 1984) or potentiate (Kasperek and Snider, 1987) the exercise activation of branched chain oxoacid dehydrogenase. These two mechanisms may account for an increased accumulation of NH_4+ and subsequent urea concentrations would increase.

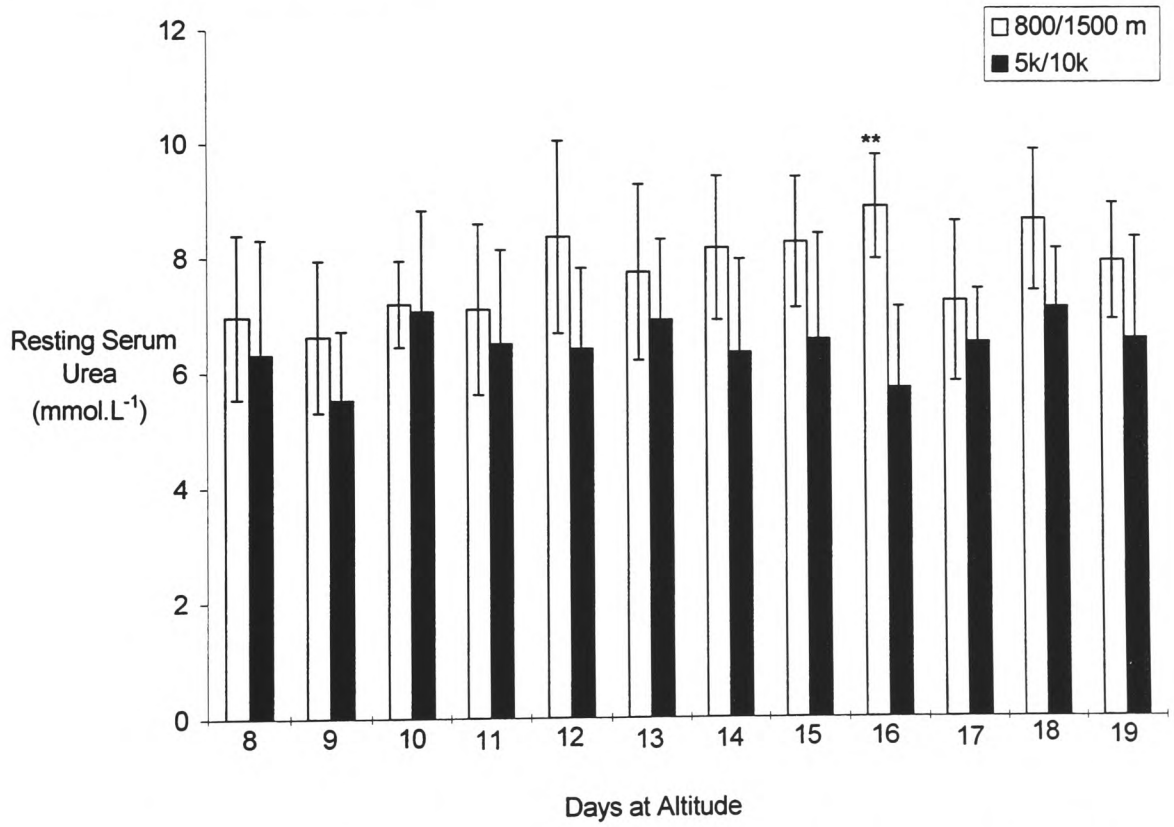


Figure 4.13 Resting Serum Urea Concentration at Altitude: A Comparison Between Male 800/1,500 m and 5 km/10 km specialists (n = 10)
Values are Mean ± SD
‡: Significantly different between groups ($P < 0.01$)

It was of interest to note that male subjects with the fastest personal best (pb) times for 1,500 m and 5 km attained the highest mean resting serum urea concentrations during the

altitude sojourn (Figures 4.14 and 4.15). The precise aetiology of this metabolic response is unknown but may represent differences in the rate of urea production and/or removal (Lemon et al 1989), the kinetics of which are affected by a variety of factors that have been previously discussed. It is quite possible that the subjects with superior pb performance times trained at a higher exercise intensity than their less elite counterparts, a prerequisite for achieving "elite" status. This would increase the production of NH_4+ and urea (Itoh and Ohkuwa, 1990 and Sahlin, 1996) possibly due to the exercise intensity-dependent activation of the branched chain oxoacid dehydrogenase (Kasparek and Snider, 1987) and an intensity-dependent breakdown of adenine nucleotides to IMP (Sahlin, 1996). As a consequence of the accelerated glycolytic flux attained during physical exercise, muscle glycogen stores may be lower in the "faster" subjects, which would further increase the production of NH_4+ and serum urea (Lemon and Mullin, 1980; Broberg and Sahlin, 1989 and Brouns et al, 1990).

This observation has not been previously documented and it is not possible in the present study to differentiate between activation of the purine nucleotide cycle an/or amino acid oxidation. However, what is apparent, is that if serum urea is validated as a reliable biochemical marker of physical stress, the more elite athletes who train at altitude may be more susceptible to overtraining.

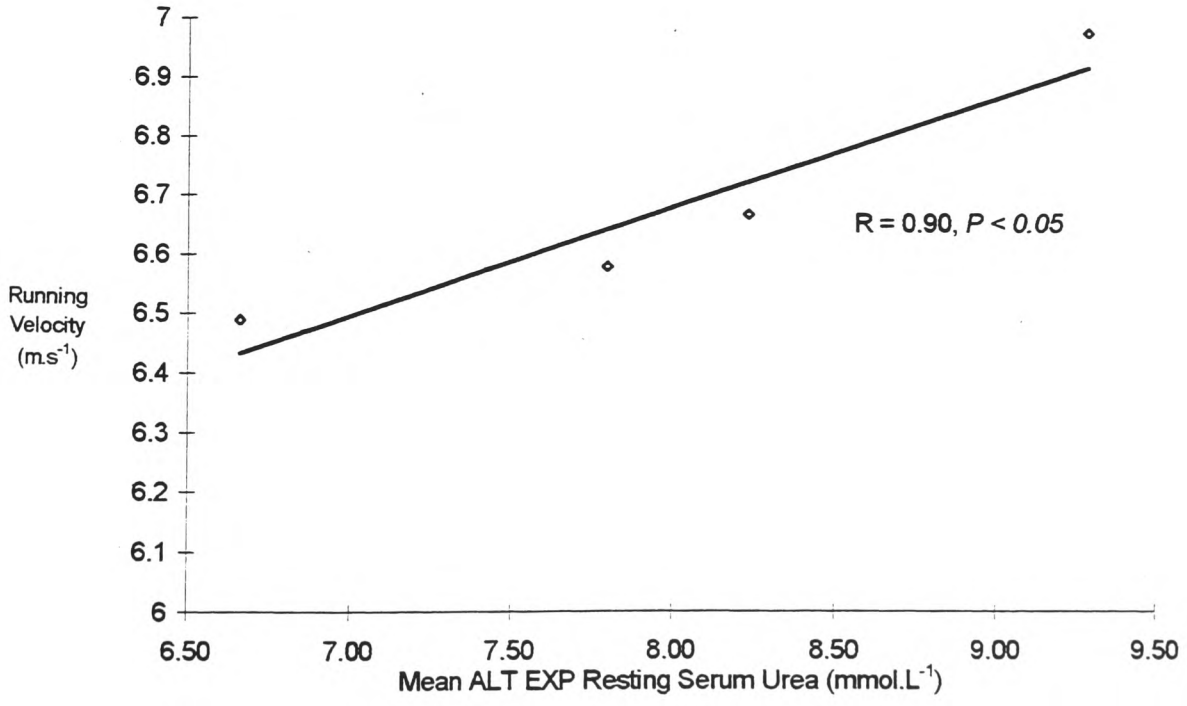


Figure 4.14 Relationship Between Resting Serum Urea Concentration at Altitude and 1,500 m Personal Best Running Velocity for Male Subjects
Values are Mean ± SD

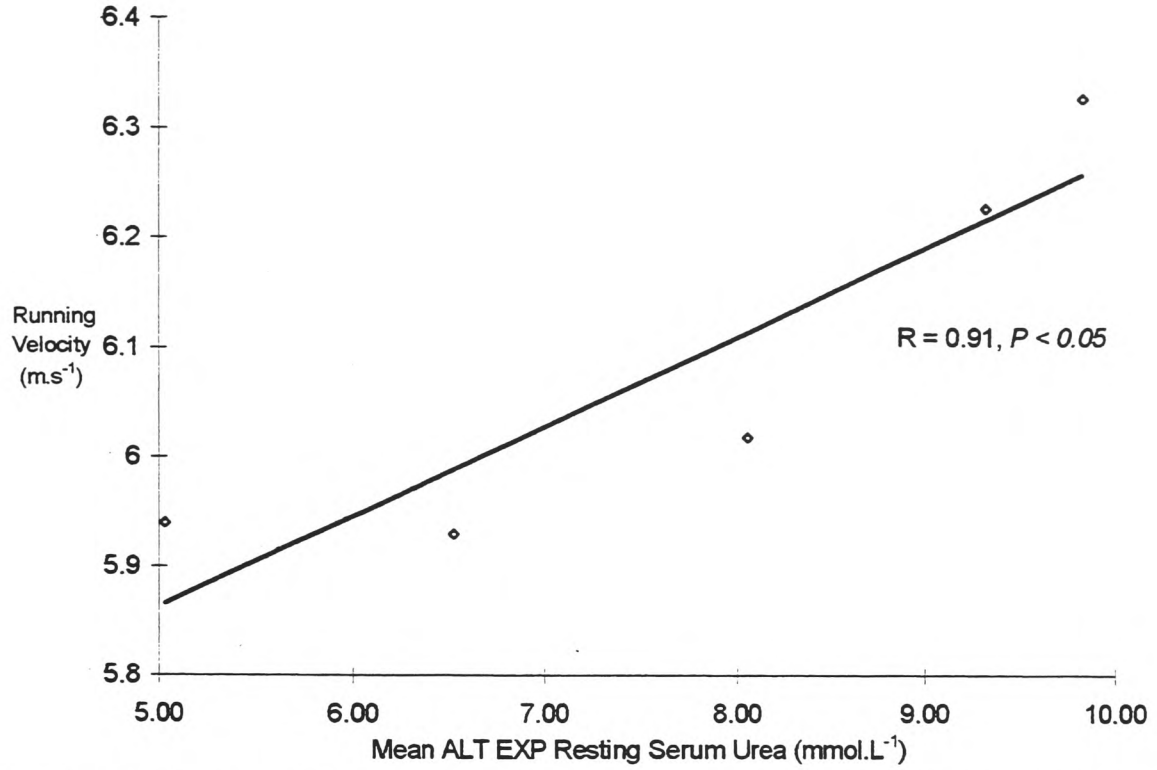


Figure 4.15 Relationship Between Resting Serum Urea Concentration at Altitude and 5 km Personal Best Running Velocity for Male Subjects
Values are Mean ± SD

4.3.3.3 Cardiovascular Adaptations

Figures 4.16 and 4.17 summarise the changes in systolic (SBP), diastolic (DBP) and mean arterial blood pressure (MABP) during the study. All subjects were normotensive acording to the The Fifth Report of the Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure (JNC V) which defines hypertension as a SBP of 140 mmHg and/or a DBP of 90 mmHg or greater. EXP group mean SBP was significantly lower on days 11, 17 and 18 at altitude ($P < 0.05$) in comparison to the pre-altitude mean value (Figure 4.16). Ten weeks of sea-level training significantly decreased CON group mean systolic pressure by 11 mmHg ($P < 0.05$). Diastolic pressures remained stable in both the EXP and CON groups throughout the experimental period (Figure 4.16). There were no significant differences in EXP group MABP between PRE (91 ± 10 mmHg), ALT (85 ± 7 mmHg) and POST (88 ± 8 mmHg) tests. However, EXP MABP was significantly lower ($P < 0.05$) on days 17 (81 ± 10 mmHg), 18 (81 ± 12 mmHg) and 19 (81 ± 6 mmHg) at altitude in comparison to the PRE EXP group mean value (Figure 4.17). There were no differences observed in CON group MABP between PRE (96 ± 10 mmHg) and POST (90 ± 7 mmHg) tests.

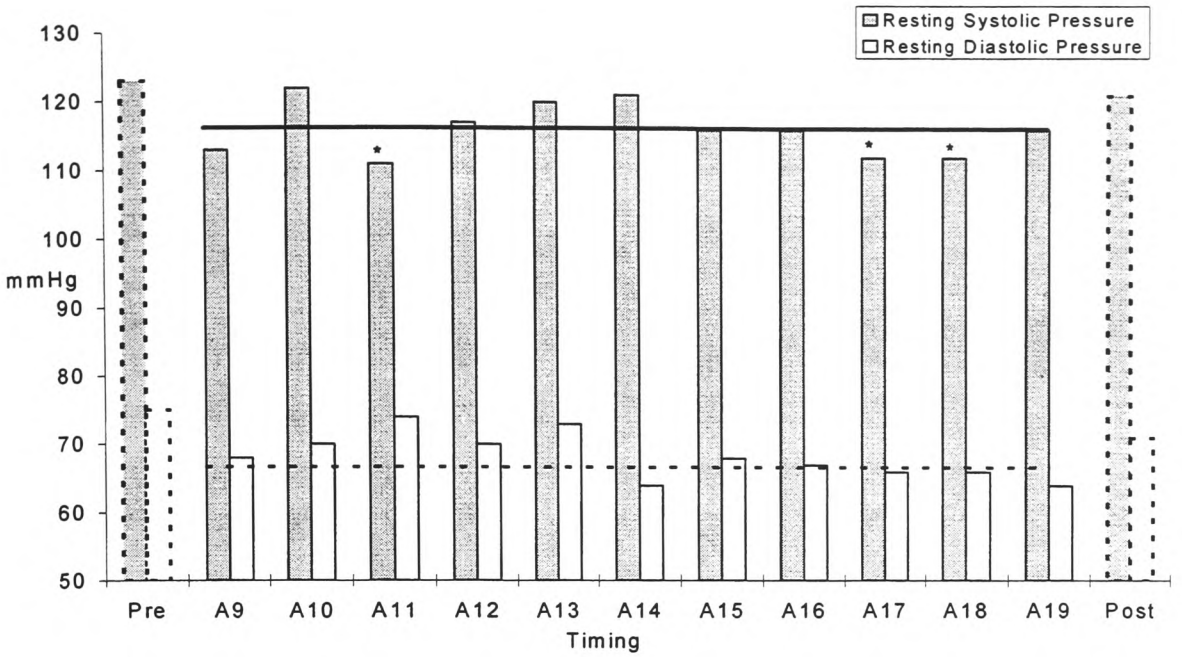


Figure 4.16 EXP Group Resting Systolic/Diastolic Pressure at Sea-Level and Altitude
Values are Mean ± SD
*: Significantly different from PRE value ($P < 0.05$)
Pre: Pre-altitude
A8 - A19: Days 8 to 19 at altitude
Post: Post altitude
Emboldened line represents mean SBP during the altitude sojourn.
Dashed line represents mean DBP during the altitude sojourn.

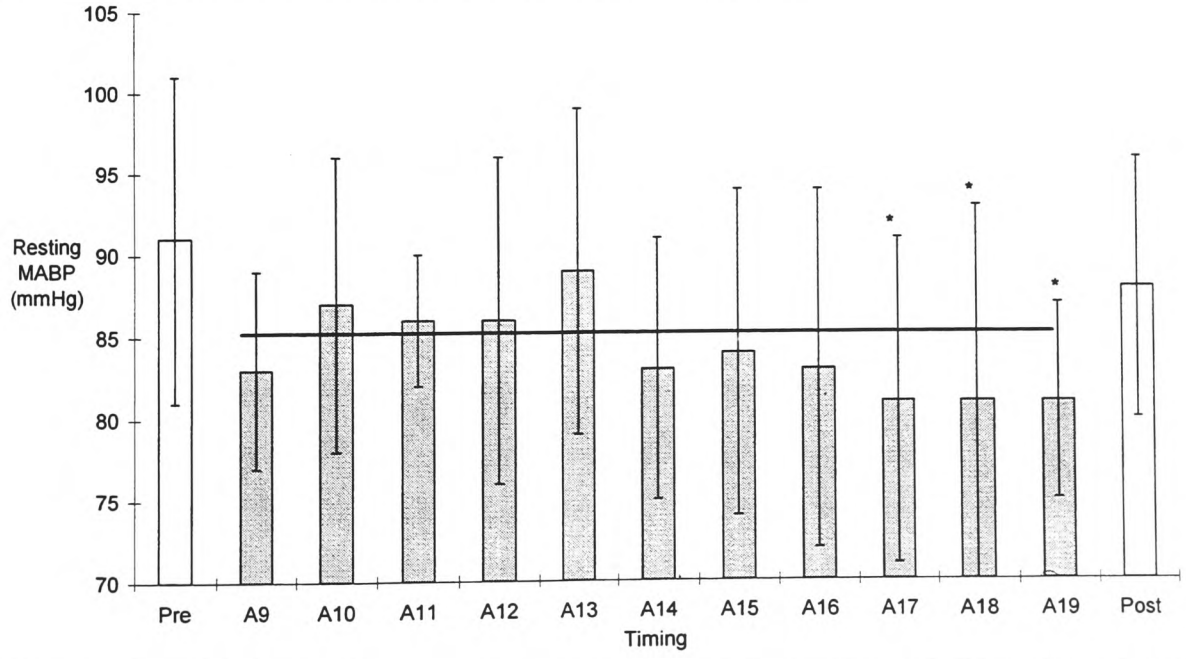


Figure 4.17 EXP Group Resting Mean Arterial Blood Pressure (MABP) at Sea-Level and Altitude
Values are Mean ± SD
*: Significantly different from PRE value ($P < 0.05$)
Pre: Pre-altitude
A8 - A19: Days 8 to 19 at altitude
Post: Post altitude
Emboldened line represents MABP during the altitude sojourn.

The effects of acute and chronic exposure to environmental hypoxia on systemic and MABP is controversial. Whilst it has been reported that exposure to 3,750 m reduces systemic blood pressure in some hypertensive patients (Penaloza, 1971), the vast majority of the research literature has demonstrated an increase at altitudes ranging between 4,000 to 4,300 m (Bender et al 1988; Morgan et al 1990 and Wolfel et al 1991). However, there is a dearth of information concerning blood pressure response at moderate altitude, similar to that encountered in the present study. The physiological mechanisms responsible for the elevation in MABP at altitude are not completely understood but may be associated with sympathetically mediated regional or systemic vasoconstriction in response to a changing CaO_2 (Wolfel et al 1991).

Thus, the significant decreases in EXP group mean systolic pressure on days 11,17 and 18 at altitude and MABP on days 17, 18 and 19 in the present study are difficult to explain. A decrease in MABP may have occurred in response to a decrease in cardiac output ($\dot{Q}$) and/or total peripheral resistance (TPR). It is unlikely that resting $\dot{Q}$ was significantly decreased at altitude as dehydration was minimised by enforcing a strict fluid intake regime. Decreases in resting $\dot{Q}$ would need to exceed 10% to cause a decrease in MABP (Tortora, 1996). It is possible that the control mechanisms of TPR may be altered at altitude. Whilst only a speculation, moderate hypoxia could increase nitric oxide (NO) production/upregulation in response to increased oxidative stress (Section 2.9.2). Recently identified as an endothelium-derived relaxing factor (EDRF), NO relaxes adjacent vascular smooth muscle and acts as a functional antagonist to sympathetic neural constriction (Green et al 1996). To this author's knowledge, there is no published research which has addressed the effects of NO production at altitude.

Figure 4.18 illustrates EXP group mean supine and standing HR on consecutive days at altitude. EXP group mean supine HR remained stable at altitude and averaged 42 ± 4 $b.min^{-1}$ (Range; 38 to 47 $b.min^{-1}$). On standing, HR increased to 63 ± 8 $b.min^{-1}$ at altitude (Range; 51 to 80 $b.min^{-1}$). Standing HR on A14 was significantly greater than the ALT 8 value ($P < 0.01$). Figure 4.19 summarises delta (Δ) HR (Standing-Supine HR) at altitude. Whilst EXP Δ HR tended to increase during the altitude sojourn these values attained significance on A14 ($P < 0.01$ vs A8) and ALT 19 ($P < 0.05$ vs A8) only. This index, which represents a modified orthostatic test was developed by Czajkowski (1982) who monitored 10 elite female cross country skiers whilst training at altitude. He demonstrated

that the ΔHR of *fit and healthy* subjects ranged between 4-8 b.min^{-1}. Any significant increase beyond this range was powerfully associated with underperformance, possibly as a consequence of overtraining. Whilst the author did not present a physiological rationale for his findings, alterations in orthostatic function may represent autonomic or hormonal dysregulation of the parasympathetic and/or sympathetic nervous systems. Israel (1976) was the first investigator to differentiate between the sympathetic and parasympathetic types of overtraining. Sympathetic overtraining constitutes a prolonged stress response, characterised by an increased sympathetic activity in the resting state, whereas parasympathetic overtraining is a more advanced condition which represents exhaustion of the neuroendocrine system (Stone et al 1991). The EXP group mean ΔHR in the present study averaged 21 ± 9 b.min^{-1} (Range: 12-40 b.min^{-1}) which, based on Czajkowski's (1982) interpretation of this data, would suggest that all subjects were in an overtrained state. However, there was no association between ΔHR and serum urea at altitude (r = 0.31, NS), possibly due to the transient kinetics of urea metabolism (Lemon, 1989). It would appear that further research is required to establish which, if any of these are the most valid indicators for the prevention of overtraining at altitude.

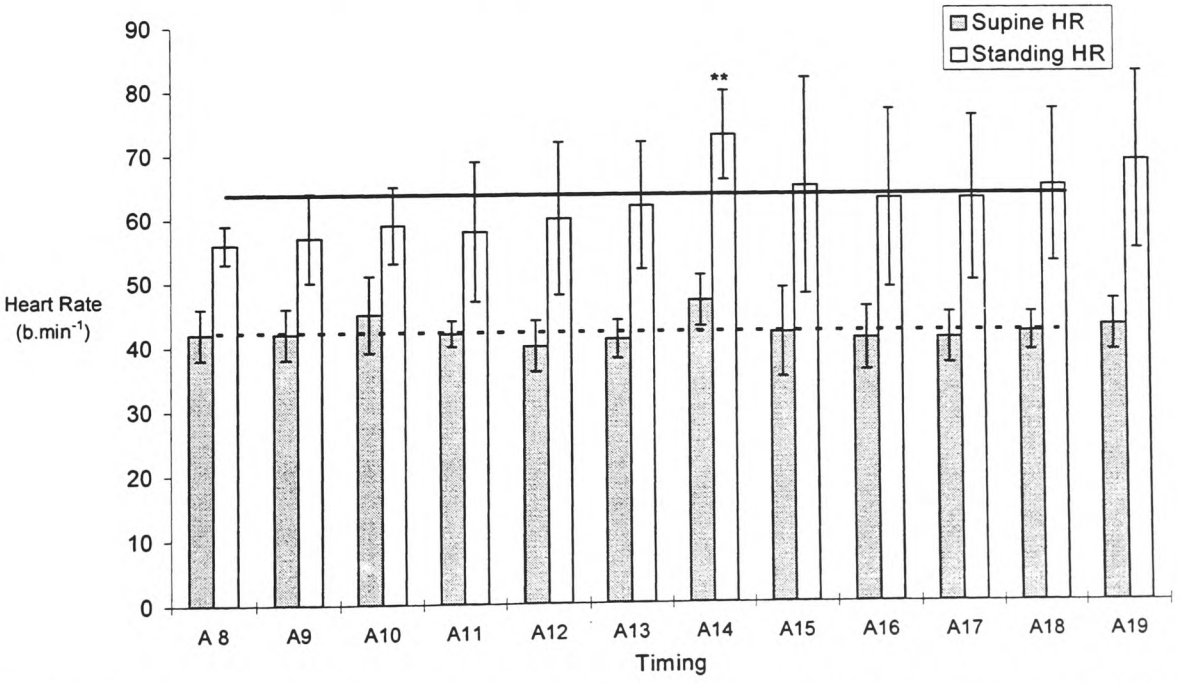


Figure 4.18 Supine and Standing Heart Rate (HR) at Altitude During a Modified Orthostatic Stress Test

Values are Mean ± SD

**: Significant difference between A8 *(P < 0.01)*

A8 - A19: Days 8 to 19 at altitude

Emboldened line represent mean standing HR during the altitude sojourn

Dashed line represent mean supine HR during the altitude sojourn

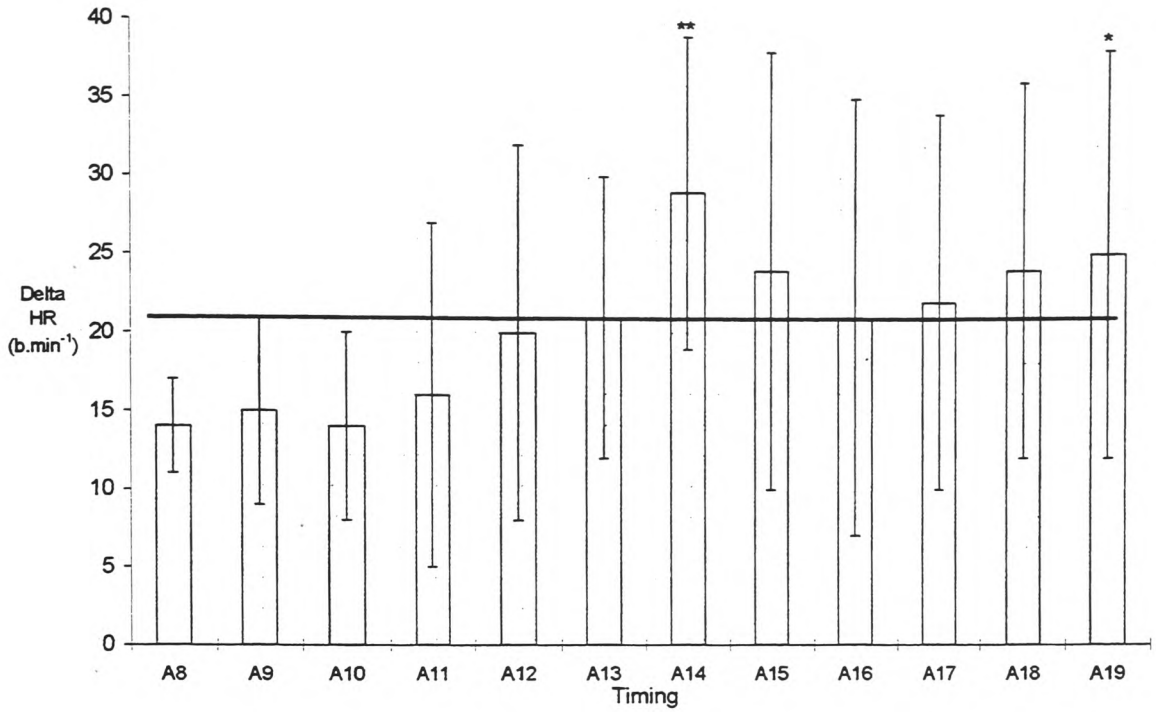


Figure 4.19 Modified Orthostatic Stress Test at Altitude
Values are Mean ± SD
*: Significantly different from ALT 8 value ($P < 0.05$)
**: Significantly different from ALT 8 value ($P < 0.01$)
A8 - A19: Days 8 to 19 at altitude
Emboldened line represents mean delta HR during the altitude sojourn

4.3.3.4 Respiratory Adaptations

All indices of pulmonary function demonstrated that the subjects' lungs were healthy and there was no evidence of any obstructive or restrictive lung disease. CON group mean lung volumes and flow rates tended to be greater than the EXP group mean values; however, these differences did not attain statistical significance. The recorded lung volumes and flow rates were within the normal values for subjects of the same age, sex, height and weight. Altitude and/or sea-level training had no effect on pulmonary function (Table 4.12). Whilst pulmonary function was not assessed at altitude, 5 subjects in the EXP group experienced shortness of breath and wheezing during and following exercise which ceased following descent to sea-level. The cause of these symptoms remains unclear. Whilst the reduced P_IO_2 may have been implicated in this response, it was suggested that an allergic reaction to the pollen from the Juniper trees in combination with the dry air may also have played a contributory role (personal communication, Dr. R.Robergs, University of New Mexico, Albuquerque, USA). The pathogenesis of exercise induced asthma (EIA) or exercise induced bronchospasm (EIB) remains controversial (Lemanske, 1989 and Kyle, 1994). An

increased respiratory water loss at altitude due to an increase in minute ventilation and dry air could result in a state of transient hyperosmolality of the fluids bathing the respiratory tract. Inhalation of hypertonic saline aerosols has been demonstrated to cause bronchospasm (Schoeffel et al 1981). Other stimuli such as the pollen previously mentioned may cause bronchospasm mediator release such as histamine and neutrophil chemotactic factor of anaphylaxis, a response termed immediate hypersensitivity (Lemanske, 1989).

Table 4.12 Lung Function Data

Variable	PRE EXP	POST EXP	PRE CON	POST CON
FVC - L	4.86 ± 1.06	4.79 ± 1.13	4.90 ± 0.77	4.79 ± 0.77
FEV_1 - L	3.87 ± 0.77	3.87 ± 0.84	4.14 ± 0.55	4.07 ± 0.58
PEF - $L.min^{-1}$	8.87 ± 1.69	8.69 ± 2.17	9.88 ± 1.57	10.36 ± 1.63
$FEF_{25-75\%}$ $L.min^{-1}$	3.71 ± 0.89	3.74 ± 1.04	4.66 ± 1.04	4.48 ± 0.93
MVV $L.min^{-1}$	159 ± 35	158 ± 39	180 ± 34	185 ± 33

Values are Mean ± SD.
FVC: Forced vital capacity
FEV_1: Forced expiratory volume (1s)
PEF: Peak expiratory flow rate
$FEF_{25-75\%}$: Mid-expiratory flow rate
MVV: Maximum Voluntary Ventilation
PRE: Pre-altitude
POST: Post-altitude
EXP: Altitude group (n = 9)
CON: Sea-level group (n = 9)

4.3.3.5 Immune Function

In vivo or *in vitro* immunocompetence was not assessed in the present study. However, the fact that five male and 2 female subjects (n = 7) in the EXP group contracted an upper respiratory tract infection (URTI) and diarrhoea between days 15 to 19 at altitude may suggest a possible link between immunosupression and an increased susceptibility to developing an infectious illness. Physical symptoms included; sore throat, coughing, upper and lower limb soreness, sinusitis, headache and general lethargy.

Physical symptoms appeared to be most pronounced in two male subjects who had contracted an URTI. It was apparent that they were also suffering from cervical lymphadenopathy and a low grade fever at altitude. Delta serum urea (ALT mean value minus PRE value) for these subjects was greater than the EXP group mean (2.64 $mmol.L^{-1}$

vs 1.19 ± 0.68 mmol.L^{-1} respectively), whereas other indices of cardiorespiratory and metabolic function were comparable. It was also apparent that they were sleeping for up to 20 h per day. Contrary to the author's advice, they continued to train for 30 minutes each day at a HR of 120-130 b.min^{-1} during the final week at altitude.

Symptoms persisted following return to sea-level and one male subject (800/1,500 m specialist) reported to the laboratory complaining that his general health and running performance had deteriorated rapidly. Despite his general malaise, he had attempted an elite 800 m race within the first week of descent to sea-level and noted that his time was 10 s slower than his personal best. A low-grade fever and cervical lymphadenopathy persisted 14 days following return to sea-level and haematological data indicated a modest leucocytosis, absolute lymphocytosis and a positive Paul-Bunnell test confirmed the diagnosis of infectious mononucleosis (Bailey et al 1997). The other male subject (5,000 m specialist) suffered similar symptoms and was also diagnosed with infectious mononucleosis after 3 weeks at sea-level.

A review by Meehan (1987) has suggested that the additive stimulus of hypoxia per se may be implicated in adverse changes in immune function, in particular suppression of cell-mediated immunity. Thus, it is possible that altitude training may increase a subject's susceptibility to becoming overtrained, the potential implications of which have been largely ignored by the scientific literature (Bailey and Davies, 1997). Whether hypoxia per se is responsible for adverse changes in immune function in the present study can remain only speculative. However, it is possible that the reduced P_IO_2 and/or the continuation of physical exercise at altitude despite an URTI may have precipitated an adverse immune response resulting in infectious mononucleosis in two male subjects.

4.3.4 Laboratory Measurements

The following sections will present and discuss the physiological responses to a standardised submaximal treadmill test. Three male and two female subjects in the EXP group were excluded from the overall analyses due to injury or illness. A female athlete in the CON group was also excluded due to injury.

4.3.4.1 Environmental Conditions and Fluid Loss

Table 4.13 summarises the changes in ambient temperature, relative humidity and barometric pressure during laboratory testing. Despite the stability of environmental conditions, sweat rate and subsequent fluid loss were significantly greater ($P < 0.05$) during POST testing in the CON group only (Table 4.14). An increased volume of sweat has been demonstrated in heat acclimatised subjects (Maughan, 1994 and Maughan and Shireffs, 1997). Whether the data in the present study represent an improvement in the CON group's thermoregulatory response to submaximal exercise can remain only speculative. However, the data would have been more conclusive if the sodium and chloride content of sweat had been determined in the present study. A decrease in these electrolytes has been observed during heat acclimatisation, a protective mechanism which serves to defend extracellular volume (Kobayashi et al, 1980 and Maughan and Shireffs, 1997).

Table 4.13 Environmental Conditions During Laboratory Testing

Variable	PRE	POST
Temperature (°C)	20.2 - 21	18.8 - 23
Relative Humidity (%)	35 - 55	33 - 48
Barometric Pressure (mmHg)	736 - 776	756 - 760

Values are Ranges
PRE: Pre-altitude
POST: Post-altitude

Table 4.14 Fluid Loss and Sweat Rate During Submaximal Exercise

Group/Timing	Fluid Loss (ml)	Sweat Rate ($ml.min^{-1}$)*
PRE EXP	788 ± 234 (450 - 1200)	18.8 ± 5.6 (10.7 - 28.6)
POST EXP	856 ± 295 (500 - 1350)	20.4 ± 7.0 (11.9 - 32.1)
PRE CON	628 ± 217 (350 - 950)	15.1 ± 5.0 (8.5 - 22.6)
POST CON	722 ± 275† (350 - 1150)	17.2 ± 6.5† (8.5 - 27.4)

Values are Mean ± SD
*: Sweat rate calculated for 42 minutes of exercise
†: Significantly different from PRE value ($P < 0.05$)
PRE: Pre-altitude
POST: Post-altitude
EXP: Altitude group (n = 8)
CON: Sea-level group (n = 9)

4.3.4.2 Metabolic Adaptations

A decreased lactacidosis is of functional significance as lactate formation is implicated in the fatigue process. Increases in $[La^-]_B$ are related to fatigue either through an increased breakdown of intramuscular glycogen or through proton induced acidosis which inhibits both biochemical and physiological processes (Sahlin, 1994,).

There were no significant differences observed in CON group mean $[La^-]_B$ between PRE and POST tests (Table 4.15 and 4.16). However, POST EXP group mean $[La^-]_B$ was significantly lower ($P < 0.05$) following return to sea-level (POST) during all 5 stages of the treadmill test. The decrease in EXP group mean $[La^-]_B$ between PRE and POST tests averaged 0.63 $mmol.L^{-1}$ ($P < 0.05$). Whether the decrease in $[La^-]_B$ was due to changes in production, release or removal is not completely understood and was not addressed in the present study due to the invasive nature of femoral arterial catheritisation.

Table 4.15 Changes in Whole Blood Lactate ($[La^-]_B$) During Individual Stages of a Standardised Submaximal Treadmill Test

Timing	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
PRE EXP	0.78 ± 0.32	1.13 ± 0.24^	1.74 ± 0.34^	3.50 ± 0.63^	6.30 ± 1.02^
POST EXP	0.45±0.13†	0.65±0.24†	1.23±0.34†^	2.78±0.74†^	5.17±1.51^†
PRE CON	0.70 ± 0.30	1.01 ± 0.43^	1.54 ± 0.32^	3.58 ± 0.62^	6.43 ± 0.89^
POST CON	0.38 ± 0.26	0.63 ± 0.21^	1.30 ± 0.33^	3.28 ± 0.70^	6.49 ± 0.97^

Values are Mean ± SD
†: Significantly different from within group PRE value ($P < 0.05$)
^: Significantly different from preceding value ($P < 0.05$)
PRE: Pre-altitude
POST: Post-altitude
EXP: Altitude group (n) = 7
CON: Sea-level group (n) = 8

Table 4.16 Group Mean Whole Blood Lactate ($[La^-]_B$) Response During a Submaximal Treadmill Test

Group	PRE	POST
EXP	2.69 ± 0.37	2.06 ± 0.54†
CON	2.65 ± 0.24	2.42 ± 0.37

Values are Mean ± SD
†: Significantly different from PRE value ($P < 0.05$)
PRE: Pre-altitude
POST: Post-altitude
EXP: Altitude group (n = 7)
CON: Sea-level group (n = 8)

To date, only 2 investigations have attributed the potentiating effects of hypoxic training to a reduced lactacidosis yet they are both characterised by significant experimental deficiencies (Terrados et al 1988 and Inger and Myhre, 1992). Terrados et al. (1988) demonstrated a significant decrease (-30%, $P < 0.05$ vs pre-altitude) in submaximal $[La^-]_B$ following 3 to 4 weeks of intermittent hypobaric hypoxic training. No metabolic changes were noted in the normoxically trained group. However, the authors applied parametric statistical analyses to their data. It is highly unlikely that their data were normally distributed with only 4 subjects in each group (normoxic/hypoxic), and it is possible that non-parametric tests may have yielded non-significant results. Despite the statistical

limitations of this study, the authors attributed the decreased lactacidosis to significant decreases ($P < 0.05$) in glycolytic enzyme activity (phosphofructokinase and lactate dehydrogenase) and increases in muscle capillarisation. In a follow up study, the same authors demonstrated that intermittent hypoxic training (2,300 m) significantly increased ($P < 0.05$) citrate synthase activity and intramuscular myoglobin content.

Ingjer and Myhre (1992) demonstrated a significant decrease in $[La^-]_B$ at 90% $\dot{V}O_2max$ following 3 weeks of altitude training at 1,900 m. The authors attributed the decreased lactacidosis to an increased CaO_2 as a function of the secondary polycythaemia. However, the lack of a normoxically trained control group in the experimental design must question the validity of their findings.

The functional significance of the reduced lactacidosis observed in the present study is questionable for two reasons. Firstly, the decrease in EXP group mean $[La^-]_B$ fell within the critical difference previously determined for the measurement of $[La^-]_B$ (-23% vs critical difference of ± 41.4%, Appendix J). Secondly, as illustrated in Table 4.17, the reduced lactacidosis was not associated with any significant changes in the anaerobic threshold (θ $[La^-]_B$). Thus, it is unlikely that the decrease in $[La^-]_B$ would have translated into any performance benefits.

Table 4.17 Group Mean Oxygen Uptake (VO_2) Values at the Lactate Threshold

Group/Timing	θ $[La^-]_B$	Correlation Coefficient (*r*)
PRE EXP	3.83 ± 0.65	0.99 ± 0.00
POST EXP	3.79 ± 0.57	0.99 ± 0.00
PRE CON	3.52 ± 0.57	0.99 ± 0.00
POST CON	3.65 ± 0.61	0.99 ± 0.00

Values are Mean ± SD
PRE: Pre-altitude
POST: Post-altitude
EXP: Altitude group (n = 7)
CON: Sea-level group (n = 8)

However, there is evidence which suggests that a more severe hypoxic stimulus could potentially decrease $[La^-]_B$ following return to normoxic conditions. The fact that the hypoxic stimulus encountered in the present study was moderate cannot be ignored. Muscle buffering capacity has been shown to increase significantly in response to physical training at 2,100 to 3,600 m (Mizuno et al 1990 and Favier et al 1995). The physiological mechanisms responsible for this particular adaptation are poorly understood, but it is conceivable that an increased bodily store of $NaHCO_3$ would facilitate intracellular buffering of $[La^-]_B$ and consequently delay the onset of H^+ induced muscle fatigue (Williams, 1992).

Alternatively, chronic exposure to environmental hypoxia has been demonstrated to increase the mobilisation and utilisation of free fatty acids (FFA) during exercise, with an associated sparing of muscle glycogen (Young et al 1982, 1987 and Braun et al 1997). A shift towards predominantly fat oxidation has also been shown to persist for up to 8 days following return to sea-level (Young et al 1982; Beidlemann et al 1996 and Bigard et al 1996). However, it is unlikely that an increased oxidation of FFA was responsible for the reduced lactacidosis in the present study due to the stability of RER between PRE and POST tests (Section 4.3.4.4). Alterations in blood NH_3 metabolism (Young et al 1987), changes in β-adrenergic sensitivity of glycolysis (Brooks et al 1992), a decrease in neuromuscular activation (Kayser et al 1994) may all contribute toward a reduction in lactate production or an increased rate of removal. A detailed analysis of these factors is discussed in Sections 2.6.3 and 2.6.5.

4.3.4.3 Cardiovascular Adaptations

There were no changes observed in EXP and CON HR between PRE and POST tests (Tables 4.18 and 4.19). By stage 5 of the treadmill test, CON group mean RPE had increased significantly from 16 ± 2 during PRE testing to 17 ± 1 ($P < 0.05$) by the POST test.

Table 4.18 Heart Rate (HR) and Ratings of Perceived Exertion (RPE) During Individual Stages of a Submaximal Treadmill Test

Variable	Timing	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
HR	PRE EXP	140 ± 10	151 ± 11^	162 ± 9^	173 ± 8^	180 ± 10^
($b.min^{-1}$)	POST EXP	137 ± 10	148 ± 11^	162 ± 9^	173 ± 9^	181 ± 7^
	PRE CON	140 ± 7	154 ± 7^	166 ± 7^	178 ± 4^	186 ± 8^
	POST CON	140 ± 5	155 ± 6^	167 ± 7^	178 ± 4^	186 ± 6^
RPE	PRE EXP	9 ± 2	11 ± 1^	13 ± 1^	15 ± 1^	17 ± 2^
	POST EXP	8 ± 2	10 ± 2^	12 ± 2^	14 ± 1^	16 ± 1^
	PRE CON	10 ± 1	11 ± 1^	12 ± 2^	14 ± 1^	16 ± 2^
	POST CON	10 ± 1	11 ± 1^	12 ± 1^	14 ± 1^	17 ± 1^†

Values are Mean ± SD
†: Significantly different from PRE value ($P < 0.05$)
^: Significantly different from preceding value ($P < 0.05$)
PRE: Pre-altitude
POST: Post-altitude
EXP: Altitude group (n = 7)
CON: Sea-level group (n = 8)

Table 4.19 Group Mean Heart Rate (HR) and Ratings of Perceived Exertion (RPE) During a Submaximal Treadmill

Variable	Group	PRE	POST
HR ($b.min^{-1}$)	EXP	161 ± 9	160 ± 9
	CON	165 ± 6	165 ± 5
RPE	EXP	13 ± 1	12 ± 2
	CON	13 ± 1	13 ± 1

Values are Mean ± SD
No significant differences between within group means ($P > 0.05$)
PRE: Pre-altitude
POST: Post-altitude
EXP: Altitude group (n = 7)
CON: Sea-level group (n = 8)

Figure 4.20 illustrates the relationship between RPE and HR for each stage of the submaximal treadmill test. This figure contains pooled PRE and POST data for both EXP and CON groups. Group mean data points are displaced to the right of the line of identity (denoted as X) which would suggest that both groups underperceived exercise intensity by a HR of approximately 30 b.min^{-1}.

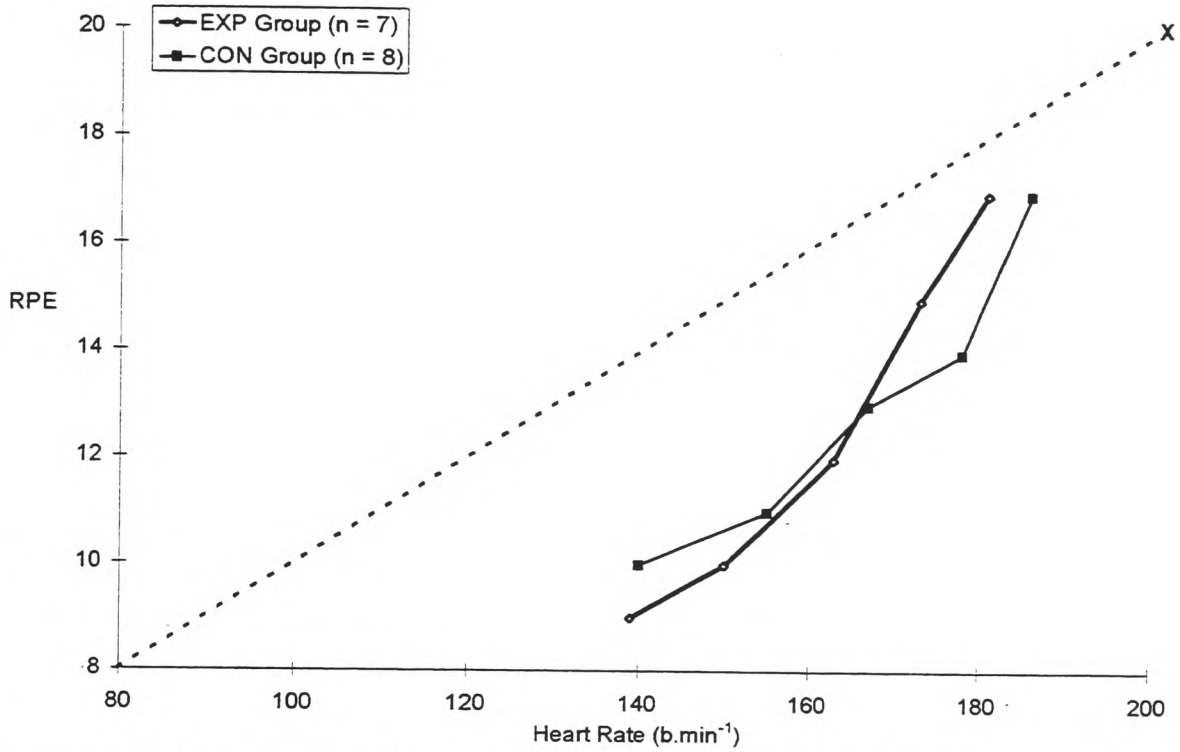


Figure 4.20 Relationship Between Submaximal Heart Rate (HR) and Ratings of Perceived Exertion (RPE)
Values are Mean
Each data point represents pooled PRE and POST group means during each treadmill stage.

The underperception of exercise intensity is a common observation particularly amongst elite male athletes training at sea-level (personal observations, British Olympic Medical Centre, UK). Chronic exposure to 4,300 m has been demonstrated to exacerbate this response, possibly due to a decrease in $[La^-]_B$ or ammonia following acclimatisation (Young et al 1982 and Young et al 1987). Whether this adaptation serves any useful purpose is unclear. Alternatively, it may increase a subject's susceptibility to becoming overtrained when challenged with an additional stress such as environmental hypoxia. This may provoke adverse changes in immune function in particular during the early stages of acclimatisation. Immunosupression combined with subsequent exposure to an infectious agent of sufficient virulence could potentially result in illness. However, there is no physiological evidence to support this hypothesis in the present study.

4.3.4.4 Respiratory Adaptations

A summary of the respiratory responses during the submaximal treadmill test is presented in Tables 4.20 and 4.21. No differences were observed in submaximal $\dot{V}O_2$ expressed in either absolute or relative terms between PRE and POST tests. A combination of submaximal $\dot{V}O_2$ at a fixed work output and the lactate threshold (θ $[La^-]_B$) are sensitive indicators of exercise performance within a homogenous group of trained subjects (Morgan and Crab, 1992 and Joyner, 1991). The fact that these parameters did not change between PRE and POST tests in the EXP group strongly suggests that altitude training did not improve running economy, despite the decreased $\dot{V}_E$ ($P < 0.05$ vs PRE) observed during the fifth stage of the treadmill test. However, an equivalent training programme conducted at sea-level was associated with increased respiratory stress towards the latter stages of the treadmill test. CON group mean $\dot{V}_E$ significantly increased during stages 3 and 4 ($P < 0.05$ vs PRE) and $\dot{V}_E/\dot{V}O_2$ was also significantly increased ($P < 0.05$ vs PRE) during stage 4 of the treadmill test.

Substrate utilisation during exercise was not altered following return to sea-level as evidenced by an unchanged RER between PRE and POST tests. However, changes in RER have been noted following altitude training at more extreme altitudes. Young et al. (1982) demonstrated a significant reduction in exercise RER ($P < 0.05$ vs pre-altitude) within 48 h at sea-level following 18 days of training at 4,300 m. The authors attributed the decrease in RER to an enhanced lipolysis and utilisation of free fatty acids (FFA) which resulted in a decreased $[La^-]_B$ and a muscle glycogen sparing effect.

Table 4.20 Respiratory Responses to Individual Stages of a Submaximal Treadmill Test

Variable	Timing	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
$\dot{V}O_2$	PRE EXP	2.85 ± 0.43	3.31 ± 0.50	3.73 ± 0.67	4.23 ± 0.76	4.55 ± 0.89
($L.min^{-1}$)	POST EXP	2.89±0.46	3.27 ± 0.50	3.70 ± 0.54	4.15 ± 0.58	4.43 ± 0.66
STPD	PRE CON	2.61±0.35	3.01 ± 0.43	3.47 ± 0.53	3.94 ± 0.61	4.30 ± 0.69
	POST CON	2.69±0.49	3.09 ± 0.50	3.52 ± 0.57	3.98 ± 0.64	4.23 ± 0.78
$\dot{V}O_2$	PRE EXP	43.9 ± 3.2	50.9 ± 4.1	57.3 ± 6.7	65.1 ± 7.8	70.0 ± 9.7
$ml.kg^{-1}min^{-1}$	POST EXP	44.6 ± 3.9	50.5 ± 4.1	57.0 ± 4.1	64.0 ± 4.4	68.4 ± 5.9
STPD	PRE CON	42.3 ± 3.1	48.7 ± 3.1	56.0 ± 3.8	63.7 ± 4.3	69.5 ± 5.0
	POST CON	43.9 ± 4.9	50.4 ± 4.2	57.6 ± 4.6	65.0 ± 5.0	69.1 ± 7.0
$\dot{V}O_2$	PRE EXP	124 ± 11	144 ± 13	163 ± 21	185 ± 24	199 ± 30
$ml.kg^{0.75}.$	POST EXP	126 ± 12	143 ± 13	162 ± 13	181 ± 14	194 ± 18
min^{-1} STPD	PRE CON	118 ± 10	136 ± 11	157 ± 14	179 ± 16	195 ± 18
	POST CON	123 ± 16	141 ± 15	161 ± 16	181 ± 18	193 ± 24
$\dot{V}_E/\dot{V}O_2$	PRE EXP	19.2 ± 1.3	20.0 ± 1.4	21.5 ± 1.8	23.2 ± 3.0	25.5 ± 3.0
($L.min^{-1}$)	POST EXP	18.4 ± 1.0	19.8 ± 1.2	21.0 ± 2.3	23.1 ± 2.7	24.6 ± 4.0
STPD	PRE CON	19.6 ± 1.9	20.7 ± 2.0	21.0 ± 1.9	22.9 ± 1.2	26.0 ± 1.4
	POST CON	19.9 ± 3.6	20.7 ± 2.5	22.2 ± 2.5	24.7 ± 2.4†	27.7 ± 2.1
$\dot{V}_E$	PRE EXP	54.7 ± 9.2	66.5 ± 12.6	80.6 ± 16.9	97.2 ± 16.8	114.8 ± 19.8
($L.min^{-1}$)	POST EXP	53.5 ± 9.5	64.9 ± 11.0	77.8 ± 14.9	96.3 ± 17.8	108.5±20.3†
STPD	PRE CON	50.7 ± 4.0	61.7 ± 6.1	72.2 ± 7.4	89.7 ± 12.2	111.4 ± 17.9
	POST CON	52.0 ± 3.7	62.7 ± 5.2	76.9 ± 6.0†	97.2±10.8†	116.3 ± 16.3
RER	PRE EXP	0.88 ± 0.04	0.91 ± 0.03	0.95 ± 0.04	1.01 ± 0.06	1.07 ± 0.06
	POST EXP	0.87 ± 0.03	0.92 ± 0.03	0.95 ± 0.03	1.01 ± 0.03	1.07 ± 0.05
	PRE CON	0.88 ± 0.03	0.93 ± 0.01	0.95 ± 0.02	1.01 ± 0.03	1.10 ± 0.04
	POST CON	0.86 ± 0.04	0.90 ± 0.04	0.95 ± 0.03	1.02 ± 0.02	1.11 ± 0.03

Values are Mean ± SD
†: Significantly different from PRE value ($P < 0.05$)
PRE: Pre-altitude
POST: Post-altitude
EXP: Altitude group (n = 7)
CON: Sea-level group (n = 8)

Table 4.21 Group Mean Respiratory Responses to a Submaximal Treadmill Test

Dependent Variable	Group	PRE	POST
$\dot{V}O_2$ (L.min^{-1}) STPD	EXP	3.74 ± 0.64	3.69 ± 0.52
	CON	3.47 ± 0.50	3.50 ± 0.58
$\dot{V}O_2$ (ml.kg^{-1}.min^{-1}) STPD	EXP	57.5 ± 6.0	56.9 ± 3.7
	CON	56.0 ± 3.2	57.2 ± 4.7
$\dot{V}O_2$ (ml.kg$^{-0.75}$.min^{-1}) STPD	EXP	163 ± 19	161 ± 12
	CON	157 ± 12	160 ± 16
$\dot{V}_E$ (L.min^{-1}) STPD	EXP	82.8 ± 14.6	80.2 ± 14.4
	CON	77.1 ± 9.0	81.0 ± 7.9
$\dot{V}_E/\dot{V}O_2$ (L.min^{-1}) STPD	EXP	21.9 ± 1.8	21.4 ± 2.0
	CON	22.0 ± 1.5	23.1 ± 2.5
RER	EXP	0.97 ± 0.04	0.96 ± 0.02
	CON	0.97 ± 0.02	0.97 ± 0.03

Values are mean ± SD
PRE: Pre-altitude
POST: Post-altitude
EXP: Altitude group (n = 7)
CON: Sea-level group (n = 8)

4.3.4.5 Infectious Mononucleosis and Submaximal Exercise Performance

The diagnosis of infectious mononucleosis in two male subjects shortly following return to sea-level is intriguing and warrants further investigation. An investigation as an adjunct to study 1 was designed to monitor the effects of infectious mononucleosis on physiological indices of submaximal and supramaximal exercise performance in one of these subjects who was an 800/1,500 m specialist. An individualised training program was administered by a medical officer during a 5 month rehabilitation period (Dr R.Budgett, British Olympic Medical Centre, UK) and a series of medical and physiological tests were conducted to monitor recovery from this condition (Bailey et al 1997).

Infectious mononucleosis was associated with pronounced changes in $[La^-]_B$ production and removal kinetics and HR during submaximal exercise (Figure 4.21). $[La^-]_B$ increased by between 0.47 to 1.92 mmol.L^{-1} and HR was elevated by 9-14 b.min^{-1} for a given treadmill velocity. Figure 4.22 summarises the % mean changes (Viral mean value minus pre-viral mean value) for $\dot{V}_E/\dot{V}O_2$, O_2 pulse, RER and RPE during the submaximal treadmill test

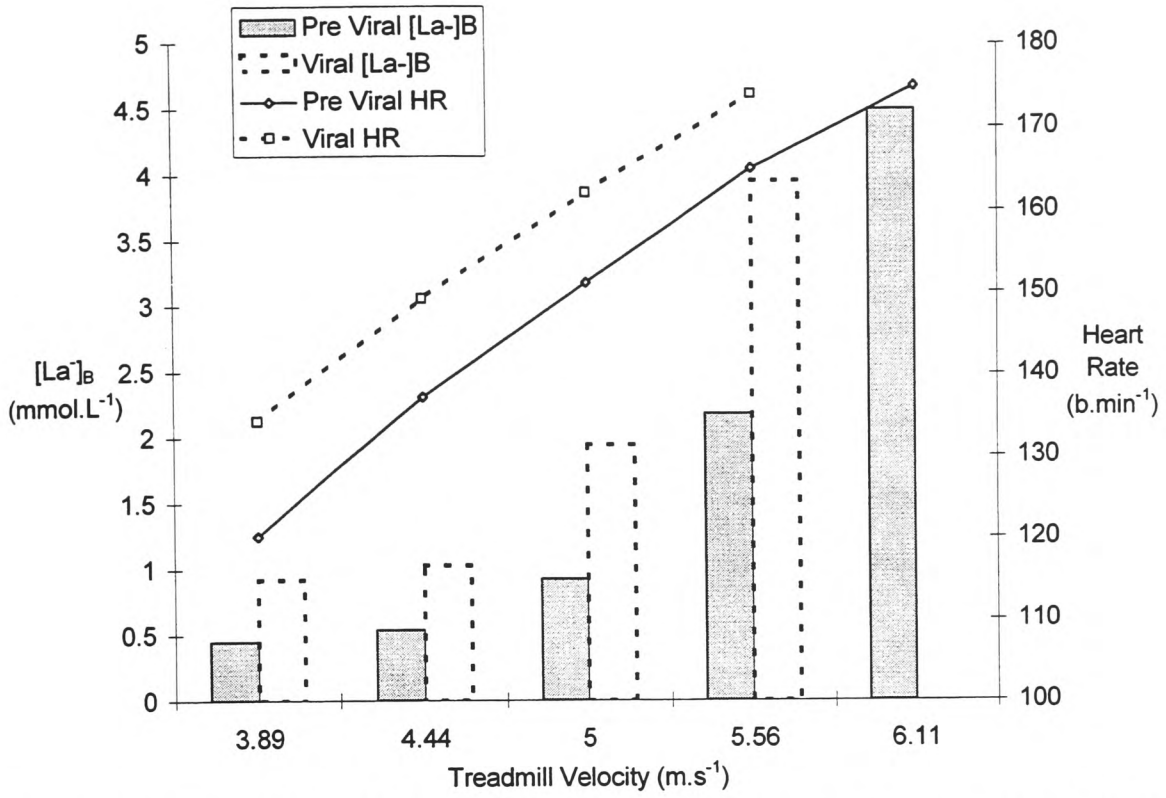


Figure 4.21 Effects of Infectious Mononucleosis on Whole Blood Lactate Concentration ($[La^-]_B$) and Heart Rate (HR) During Submaximal Exercise; A Case Study of an Elite 800/1,500 m Specialist
Values are Mean

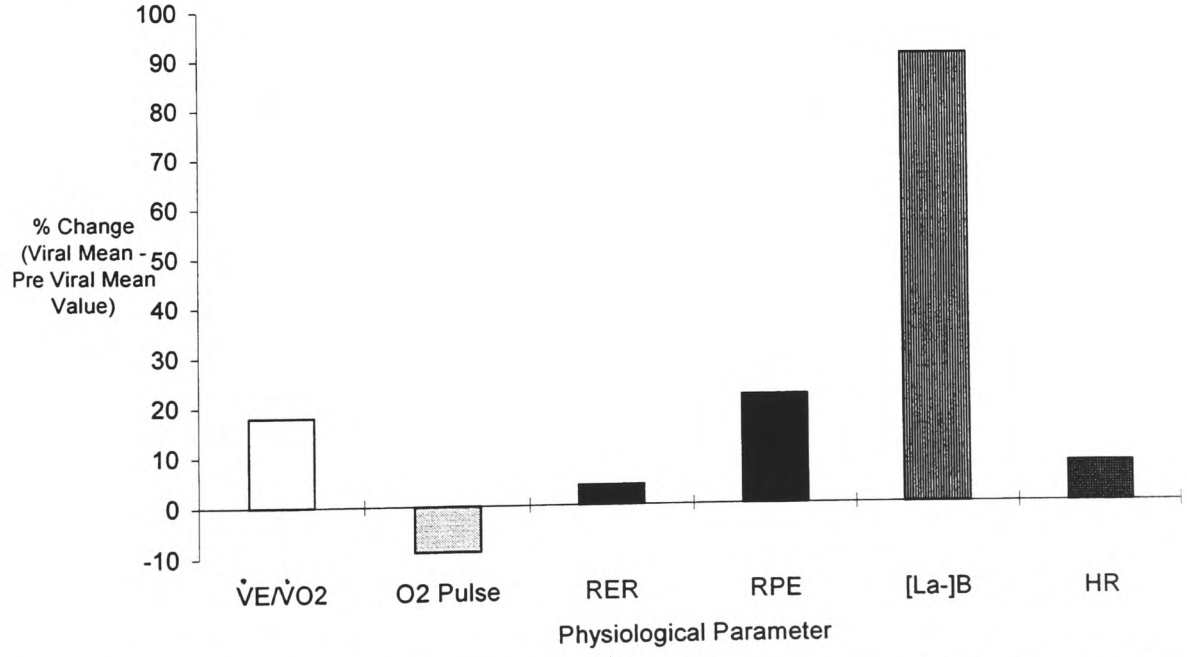


Figure 4.22 Effects of Infectious Mononucleosis on Selected Physiological Parameters During Submaximal Exercise; A Case Study of an Elite 800/1500 m Specialist.
Data represent mean values calculated during the first 4 stages of a standardised submaximal treadmill test (3.89-5.56 $m.s^{-1}$).

Chronic stimulation of the sympatho-adrenal system which has been shown to cause an accelerated glycogenolysis through activation of the rate limiting enzymes phosphorylase and phosphofructokinase (Euler, 1974) may have contributed to the lactacidosis and elevated HR observed during exercise. Infectious mononucleosis is an acute, self-limiting lymphoproliferative disease caused by the Epstein-Barr virus (EBV). Eichner (1987) has published an extensive overview of the epidemiology, pathophysiology, diagnosis and management of this disease in the athlete. Whether susceptibility to EBV invasion was triggered by hypoxia per se in the present study can remain only speculative. Assuming an incubation period of 30 to 50 days (Eichner, 1987), it is conceivable that the subjects became infected during the first few days of the altitude sojourn. The fact that subjects complained of headache, malaise, fatigue and myalgia during the first few days at altitude, symptoms that are characteristic of the first 3 to 5 day prodrome (Jenkins and Mowbray, 1991) would support this contention. Whilst there is no evidence to support the theory, it is possible that the immunosupressive effects of hypobaric hypoxia are most pronounced during the first few days at altitude when the subject is experiencing the most severe decreases in PaO_2, prior to complete haematological adaptation. Erythrocyte volume does not increase until 4 days (Klausen et al 1991) and maximum reticulocytosis occurs after approximately 8 to 10 days at altitude (Hartmann et al 1990). The additive stress of physical training would further exacerbate the $[A\text{-}a]O_2$ difference and possibly increase the host's susceptibility to an antigenic challenge.

An individualised, tapered training program, in conjunction with continuous physiological and medical assessments allowed this subject to make a full recovery from infectious mononucleosis whilst still maintaining a significant level of aerobic fitness. Ten months following diagnosis, the subject recorded a personal best indoor 1500 metres time. The outcome was less encouraging for the 5,000 m runner who had also contracted infectious mononucleosis. The severity of his symptoms precluded him from any physical training for 6 months and he did not return to competitive racing until 12 months after the initial diagnosis.

4.3.5 Track Measurements

Few altitude training studies have incorporated an event specific field test in the experimental design. The unpredictability of the environmental conditions at altitude, in particular wind velocity, may contribute to the general lack of existing information. However, it is clear from the present study, that with the exception of ambient temperature, environmental conditions remained relatively stable throughout the experimental period (Table 4.22), and thus, it is likely that the physiological responses observed were as a direct consequence of hypoxia per se.

Table 4.22 Environmental Conditions During Track Testing

Variable	PRE	ALT*	POST
Temperature (°C)	3.5 - 5.0	24.7 - 28.4	10.6 - 16.9
Relative Humidity (%)	30 - 46	2 - 10	38 - 51
Barometric Pressure (mmHg)	758 - 761	635 - 640	749 - 764
Wind Velocity ($m.sec^{-1}$)	1.7 - 2.0	2.2 - 3.0	1.9 - 2.2

Values are reported as ranges
*: Measurements conducted on day 16 at 1,500 m
PRE: Pre-altitude
ALT: Altitude
POST: Post-altitude

The physiological responses to a standardised track session conducted at sea-level and altitude are summarised in Tables 4.23 to 4.27. A total of 7 subjects (EXP group = 5 / CON group = 2) were forced to miss a track session due to illness or injury and were thus excluded from the overall analyses.

4.3.5.1 Metabolic Adaptations

There were no significant differences observed in EXP or CON group mean $[La^-]_B$ between PRE, ALT and POST testing (Table 4.23). The $\Delta[La^-]_B$ (POST - PRE group mean value) was not significantly different between EXP (-0.23 ± 1.18 $mmol.L^{-1}$) and CON (-0.58 ± 0.35 $mmol.L^{-1}$, NS) groups (Table 4.24).

Table 4.23 Changes in Whole Blood Lactate Concentration ($[La^-]_B$) During Individual Track Repetitions

Variable	Group/Timing	Repetition 1	Repetition 2	Repetition 3	Repetition 4
Rep $[La^-]_B$	PRE EXP	5.02 ± 2.72	6.98 ± 1.55	7.56 ± 1.01	8.65 ± 1.01^
$mmol.L^{-1}$	ALT* EXP	5.15 ± 1.25	6.62 ± 0.80	7.07 ± 1.21	7.43 ± 1.23
	POST EXP	4.37 ± 1.28	6.52 ± 0.44	7.79 ± 0.70^	8.61 ± 0.84^
	PRE CON	4.12 ± 1.00	6.55 ± 0.76	7.46 ± 1.07	8.17 ± 1.58
	POST CON	4.67 ± 0.47	6.31 ± 0.75	6.18 ± 0.97	6.81 ± 1.24

Values are Mean ± SD
*: Measurements conducted on day 16 at 1,500 m
PRE: Pre-altitude
ALT: Altitude
POST: Post-altitude
EXP: Altitude group (n = 6)
CON: Sea-level group (n = 5)

Table 4.24 Mean Changes in Whole Blood Lactate Concentration ($[La^-]_B$) During a Standardised Track Session

Dependent Variable	Group	PRE	ALT*	POST
Rep $[La^-]_B$	EXP	7.05 ± 1.49	6.57 ± 0.96	6.82 ± 0.62
$(mmol.L^{-1})$	CON	6.58 ± 0.62	.....	5.99 ± 0.84

Values are Mean ± SD
*: Measurements conducted on day 16 at 1,500 m
PRE: Pre-altitude
ALT: Altitude
POST: Post-altitude
EXP: Altitude group (n = 6)
CON: Sea-level group (n = 5)

4.3.5.2 Cardiovascular Adaptations

Tables 4.25 and 4.26 illustrate the changes in repetition and mean HR, recovery HR and RPE. There were no significant differences in either repetition or recovery HR between PRE and POST tests for both EXP and CON groups. POST EXP HR recovered from a repetition mean value of 174 ± 3 $b.min^{-1}$ to a recovery mean value of 166 ± 7 $b.min^{-1}$, a decrease of 8 ± 6 $b.min^{-1}$. This decrease in HR was not significantly different from the PRE

EXP or ALT EXP mean values (7 ± 5 $b.min^{-1}$ and 6 ± 3 $b.min^{-1}$ respectively). There was no significant difference observed in the decrease in HR for the CON group (PRE = 5 ± 2 $b.min^{-1}$ vs POST = 10 ± 7 $b.min^{-1}$; NS).

EXP and CON group mean values for RPE remained stable between PRE and POST tests. Again, it would appear from the relationship between RPE and HR that both groups were consistently underperceiving exercise intensity.

Table 4.25 Changes in Heart Rate (HR) and Ratings of Perceived Exertion (RPE) During Individual Track Repetitions

Variable	Group/Timing	Repetition 1	Repetition 2	Repetition 3	Repetition 4
Rep HR ($b.min^{-1}$)	PRE EXP	167 ± 5	173 ± 6^	174 ± 5	174 ± 5
	ALT* EXP	165 ± 6	171 ± 8^	172 ± 8	173 ± 8
	POST EXP	171 ± 3	174 ± 3^	175 ± 3	175 ± 3
	PRE CON	181 ± 6	184 ± 3	187 ± 3	189 ± 3
	POST CON	180 ± 6	182 ± 2	184 ± 2	185 ± 2
Recovery HR ($b.min^{-1}$)	PRE EXP	160 ± 2	166 ± 1^	168 ± 2	168 ± 1
	ALT* EXP	157 ± 12	164 ± 9	167 ± 9^	169 ± 8
	POST EXP	161 ± 7	164 ± 9	169 ± 7^	170 ± 5
	PRE CON	176 ± 7	182 ± 5	183 ± 2	182 ± 5
	POST CON	169 ± 5	171 ± 6	176 ± 8	178 ± 7
RPE	PRE EXP	14 ± 2	15 ± 2	16 ± 2^	16 ± 2
	ALT* EXP	14 ± 1	17 ± 2^	17 ± 2	18 ± 2
	POST EXP	13 ± 2	14 ± 3	16 ± 2	18 ± 2
	PRE CON	13 ± 3	14 ± 2	15 ± 1	17 ± 1
	POST CON	13 ± 2	14 ± 2	15 ± 1	17 ± 2

Values are Mean ± SD
^: Significantly different from preceding value ($P < 0.05$)
*: Measurements conducted on day 16 at 1,500 m
PRE: Pre-altitude
ALT: Altitude
POST: Post-altitude
EXP: Altitude group (n = 6)
CON: Sea-level group (n = 5)

Table 4.26 Mean Cardiovascular Responses to a Standardised Track Session

Dependent Variable	Group	PRE	ALT*	POST
Rep HR ($b.min^{-1}$)	EXP	172 ± 5	170 ± 7	174 ± 3
	CON	185 ± 3	.....	183 ± 1
Recovery HR ($b.min^{-1}$)	EXP	166 ± 1	165 ± 9	166 ± 7
	CON	181 ± 3	.....	174 ± 6
RPE	EXP	16 ± 2	17 ± 2	16 ± 2
	CON	15 ± 2	.....	15 ± 1

Values are Mean ± SD
*: Measurements conducted on day 16 at 1,500 m
PRE: Pre-altitude
ALT: Altitude
POST: Post-altitude
EXP: Altitude group (n = 6)
CON: Sea-level group (n = 5)

4.3.5.3 Running Performance

In comparison to pre-altitude group mean values, EXP group mean running velocity was significantly decreased ($P < 0.05$) by the 3rd and 4th repetitions at altitude (Table 4.27). EXP group mean running velocity decreased on average 0.15 $m.s^{-1}$ ($P < 0.05$) at altitude (Table 4.28). No differences in group mean running velocity were observed between PRE and POST testing for both the EXP or CON groups.

Table 4.27 Repetition Running Velocity During a Standardised Track Session

Variable	Group/Timing	Repetition 1	Repetition 2	Repetition 3	Repetition 4
Rep	PRE EXP	5.68 ± 0.50	5.66 ± 0.50	5.65 ± 0.49	5.68 ± 0.53
Velocity	ALT* EXP	5.68 ± 0.40	5.54 ± 0.55	5.46 ± 0.56†	5.46 ± 0.54†
($m.s^{-1}$)	POST EXP	5.71 ± 0.51	5.65 ± 0.59	5.69 ± 0.53	5.73 ± 0.55
	PRE CON	5.64 ± 0.56	5.58 ± 0.57	5.63 ± 0.58	5.62 ± 0.66
	POST CON	5.66 ± 0.67	5.62 ± 0.64	5.59 ± 0.71	5.61 ± 0.71

Values are Mean ± SD
†: Significantly different between PRE and POST tests ($P < 0.05$)
*: Measurements conducted on day 16 at 1,500 m
PRE: Pre-altitude
ALT: Altitude
POST: Post-altitude
EXP: Altitude group (n = 6)
CON: Sea-level group (n = 5)

Table 4.28 Mean Running Velocity During a Standardised Track Session

Dependent Variable	Group	PRE	ALT*	POST
Rep Running velocity	EXP	5.67 ± 0.50	5.52 ± 0.51†	5.69 ± 0.55
($m.s^{-1}$)	CON	5.62 ± 0.60	.....	5.62 ± 0.69

Values are Mean ± SD
†: Significantly different from PRE value ($P < 0.05$)
*: Measurements conducted on day 16 at 1,500 m
PRE: Pre-altitude
ALT: Altitude
POST: Post-altitude
EXP: Altitude group (n = 6)
CON: Sea-level group (n = 5)

Whilst the additive stimulus of hypobaric hypoxia may have been implicated in the improved metabolic response to submaximal exercise, there were no potentiating effects observed during supramaximal performance on return to sea-level. The only significant change observed in the present study was the decrease ($P < 0.05$ vs PRE) in EXP group mean running velocity at altitude. This was probably related to the cumulative effects of either central or peripheral fatigue as velocity was shown to decrease by the 3rd and 4th repetition only. The metabolic response at altitude was particularly intriguing. Despite the decreased ambient PO_2, there were no changes observed in the lactate response at altitude. This

would therefore suggest, albeit tentatively, that $[La^-]_B$ accumulation was not the the sole mediator of fatigue at altitude. In fact, there is an emerging body of evidence which suggests that muscle metabolic fatigue *is not* the primary factor responsible for an impaired physical performance at altitude (Kayser et al 1994). Muscle biopsies taken from the vastus lateralis muscle of altitude acclimatised subjects after exhaustive cycling exercise have demonstrated that phosphocreatine stores were less depleted, less $[La^-]_B$ had accumulated, less glycogen was hydrolised, and intramuscular pH was greater compared with sea-level values (Green et al 1989, 1992). Kayser et al. (1994) investigated the link between the central nervous system (CNS) and local fatigue following 1 month of acclimatisation to 5,050 m. They demonstrated that, in contrast to findings observed at sea-level, there was no evidence of electromyographic fatigue during maximal cycling exercise at altitude and the increase in $[La^-]_B$ was less marked. These responses were normalised following the administration of 100% O_2, which suggested that the decrease in $[La^-]_B$ typically termed the lactate paradox (Section 2.5.4.3.) and fatigue at altitude originated centrally and were mediated by hypoxia. It has been suggested that local respiratory muscle fatigue may signal the CNS, thus decreasing the central drive to the locomotory muscles (Bigland-Ritchie and Vollestad, 1988). If this is the case, high ventilation rates experienced by the elite athlete even at moderate altitudes may be implicated in the genesis of central fatigue at altitude. This contention awaits further investigation.

4.4 SUMMARY

The compliance rate in the present study was significantly lower than expected as a consequence of illness or injury. As a function of the reduction in sample size, some data were not normally distributed and thus statistical evaluation was assessed by means of non-parametric ranking analyses. Therefore the conclusions made in the present study should be treated with caution.

Chronic exposure to moderate hypobaric hypoxia decreased lactacidosis during submaximal exercise 3 weeks following return to sea-level, whereas no changes were observed following an equivalent sea-level training programme. It seems unlikely that this would have improved endurance performance considering an unchanged lactate threshold and submaximal $\dot{V}O_2$. Altitude training did not improve physiological indices of supramaximal exercise performance.

Several subjects in the EXP group contracted an infectious illness, possibly mediated by the additive hypoxic stimulus. Immunosupression was most pronounced in 2 male subjects who were consequently diagnosed with infectious mononucleosis shortly following return to sea-level. The physiological mechanisms responsible for the general lack of performance potentiating effects require further investigation, in particular the potentially debilitating effects of hypoxia-induced immunosupression. These mechanisms will be investigated in a follow-up study described in the following chapter.

CHAPTER 5

STUDY 2: S.AFRICA

THE EFFECTS OF MODERATE ALTITUDE TRAINING ON PHYSIOLOGICAL INDICES OF MAXIMAL AND SUPRAMAXIMAL PERFORMANCE

5.1 INTRODUCTION

The findings of Study 1 questioned previous conclusions formulated by other hypoxic training studies. However, Study 1 was characterised by a small sample size and thus statistical treatment of the data were limited. An increase in sample size and corresponding power of the test (Altman, 1991) would overcome this limitation, and maximise the chances of detecting any significant treatment effects. This could not be achieved in the present study due to the limited number of *elite* subjects available prior to the Atlanta Olympics, USA (1996), therefore repeating certain investigations that were conducted in Study 1 would serve to stengthen our findings.

The majority of the scientific literature is biased towards optimising the theoretically beneficial aspects of altitude acclimatisation which facilitates systemic O_2 transport, whereas few studies have considered the potentially negative or harmful effects of a prolonged hypoxic exposure (Bailey and Davies, 1997). This is intriguing given the overwhelming number of studies that have failed to support the potentiating effects of altitude training following return to sea-level.

Whilst it was clear from Study 1 that hypobaric hypoxia was *associated* with a moderate but significantly reduced lactacidosis during submaximal exercise, there was also evidence of an altered immune response. Several subjects who sojourned to altitude contracted an infectious illness and two subjects were diagnosed with infectious mononucleosis shortly following return to sea-level. Whilst there was no direct evidence to suggest that hypoxia per se was responsible for this response, there is some evidence of hypoxia-mediated immunosupression at altitude in sedentary subjects (Meehan, 1987). However, to this author's knowledge, there is no research which has addressed the immunomodulatory roles of hypobaric hypoxia in a cohort of highly conditioned subjects, who, by virtue of their intensive training programmes, may be even more susceptible to an antigenic invasion by microbacterial agents, in particular viruses (Nieman et al 1990 and Pedersen, 1996).

Since glutamine is an important substrate required by key cells of the immune system, (Ardawi and Newsholme, 1985) the present study was designed to investigate the effects of 4 weeks of moderate altitude training (~1,640 m) on glutamine metabolism and the potential implications for both the fitness and health of the elite competitor. The potentially

modulating roles of subject iron status and timing of descent to sea-level on maximal and supramaximal indices of exercise performance were also studied. The present study was designed as a follow-up to study 1, conducted at a similar altitude and at the same time of year to control for seasonal variation (Reilly, 1994).

5.2 METHODS

5.2.1 Selection of Subjects

Twenty two male and seven female subjects (n = 29) were recruited from a pool of International standard distance runners who were born and raised at or near sea-level. Eleven male and three female subjects (n = 14) had also participated in study 1. Potential subjects were excluded from participation if they were anaemic or suffering from an injury or a viral infection. Subject recruitment was conducted at Loughborough University, UK, in conjunction with the National Endurance Coach for 5 km to 10 km.

5.2.2 Subject Characteristics

Anthropometric, dietary and track running performance data are presented in Tables 5.1 and 5.2.

Table 5.1 Anthropometric and Dietary Characteristics of Subjects (n = 29)

Dependent Variable	Group Mean ± SD *(Range)*
Age (Years)	23 ± 4
	(18 - 34)
Body Mass (Kgs)	65.8 ± 7.1
	(51.1 - 79.5)
Stature (m)	1.76 ± 0.08
	(1.61 - 1.90)
Body Fat (%)	10.2 ± 4.6
	(3.8 - 20.4)
Systolic Blood Pressure (mmHg)	117 ± 11
	(100 - 140)
Diastolic Blood Pressure (mmHg)	69 ± 10
	(50 - 90)
Forced Vital Capacity (L)	5.19 ± 0.78
	(3.59 - 6.41)
Daily Calorific Intake (KCal)	2999 ± 762
	(1863 - 4634)
Daily Carbohydrate Intake (g.kg bwt^{-1})	7.1 ± 1.6
	(5.2 - 10.6)
Daily Carbohydrate Intake	57 ± 7
(% of Total Calorific Intake)	*(44 - 73)*

Table 5.2 Track Running Performance Data (n = 29)

Distance (m)	♂ n	♀ n	♂ Group Mean *(Range)*	♀ Group Mean *(Range)*
800*	13	3	1:51 ± 0:02 *(1:48 - 1:53)*	2:08
1,500*	12	2	3:47 ± 0:06 *(3:39 - 3:58)*	4:33 *(4:21 - 4:45)*
3,000	2	1	8:33 *(8:20 - 8:45)*	9:56
5,000	3	1	13:45 *(13:10 - 14:08)*	15:00
10,000	1	0	28:28	.....

Values are expressed in minutes : seconds
*: some subjects are classified as 800 and 1,500 m specialists (both times are included in data analysis)

Subjects were separated into two groups that were matched for track running performance. Nine males and one female (n = 10) were assigned to an altitude training group (EXP) who were to spend 4 weeks training at an altitude of ~1,640 m in Krugersdorp, S.Africa in preparation for the Olympic Games in Atlanta, USA. The remaining thirteen males and six females (n = 19) continued with their normal training programme at sea-level based at Loughborough, UK (CON). All subjects were taking oral iron supplements (200 mg per day of ferrous sulphate). Anthropometric and activity data for both EXP and CON groups are presented in Table 5.3.

Table 5.3 Group Anthropometric and Activity Data

Dependent Variable	EXP (n = 10)	(n = 19)
Age (Years)	22 ± 4	24 ± 4
Body Mass (Kgs)	68.6 ± 7.3	64.2 ± 6.6
Stature (m)	1.79 ± 0.07	1.75 ± 0.08
Body Fat (%)	9.1 ± 3.9	11.0 ± 4.9
Forced Vital Capacity (L)	5.40 ± 0.91	5.07 ± 0.71
Running Distance ($km.week^{-1}$)	99 ± 26	104 ± 30

Values are Mean ± SD.
EXP: Altitude group
CON: Sea-level group

5.2.3 Experimental Design and Protocol

An overview of the experimental design and protocol utilised in the present study is presented in Figures 5.1 and 5.2. An elite male 800 m specialist volunteered for a pilot study which was designed to assess the suitability of specific treadmill tests for the determination of maximum oxygen uptake ($\dot{V}O_{2max}$). Since the classical work of Hill (1923), this variable has been traditionally used as a laboratory measure to monitor changes in training-induced adaptations and improvements in endurance performance (Jakeman et al, 1994). However, several validated treadmill protocols are inherently dangerous and not suitable for the testing of the elite distance runner due to the high velocities achieved at $\dot{V}O_{2max}$ (personal observations, British Olympic Medical Centre, UK). For this reason, a treadmill protocol validated by Taylor et al (1955) was employed in the present study. The authors demonstrated a coefficient of reliability of 0.95 based on 69 test-retest measurements.

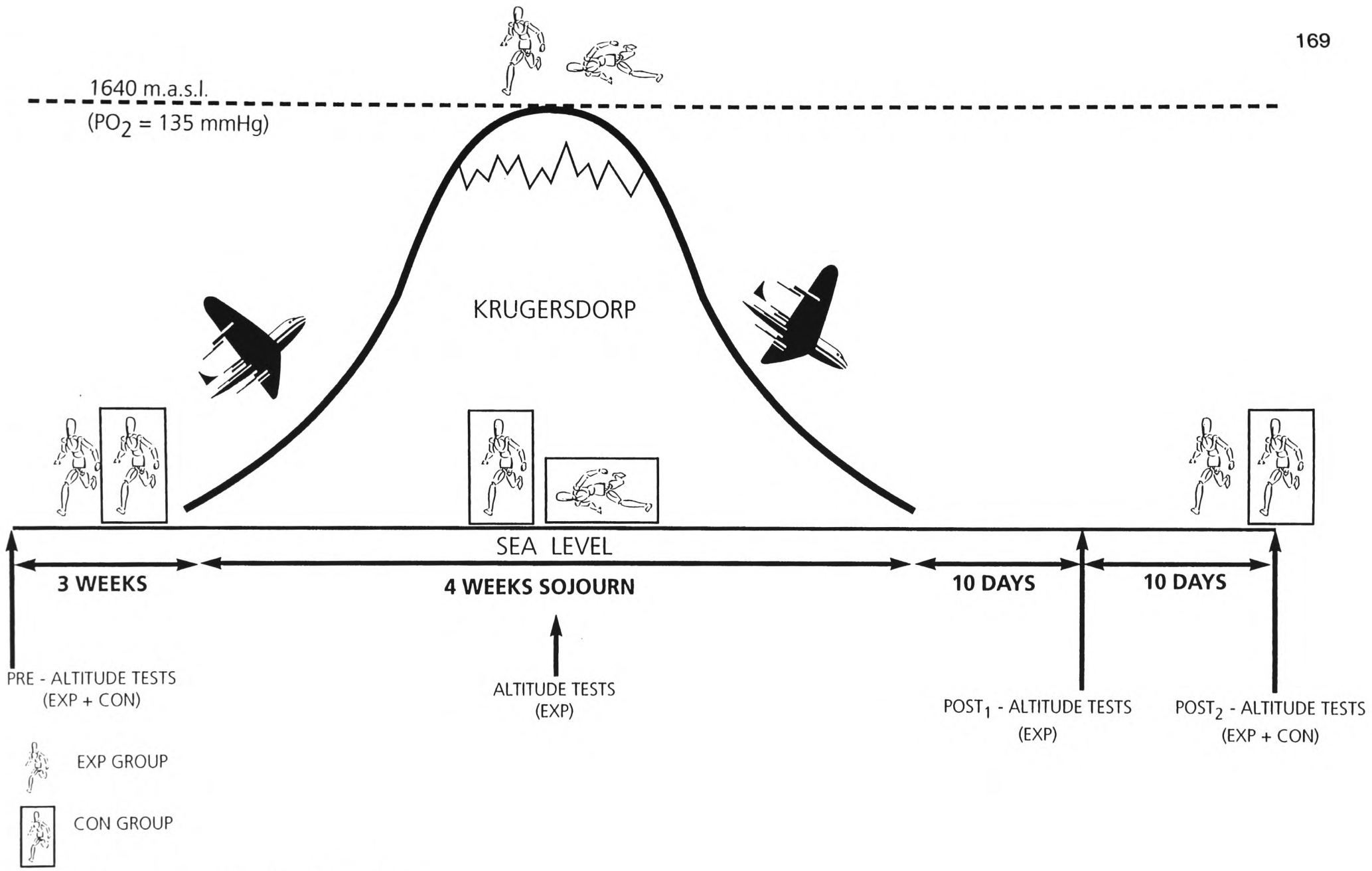


Figure 5.1 Experimental Design (S. Africa)

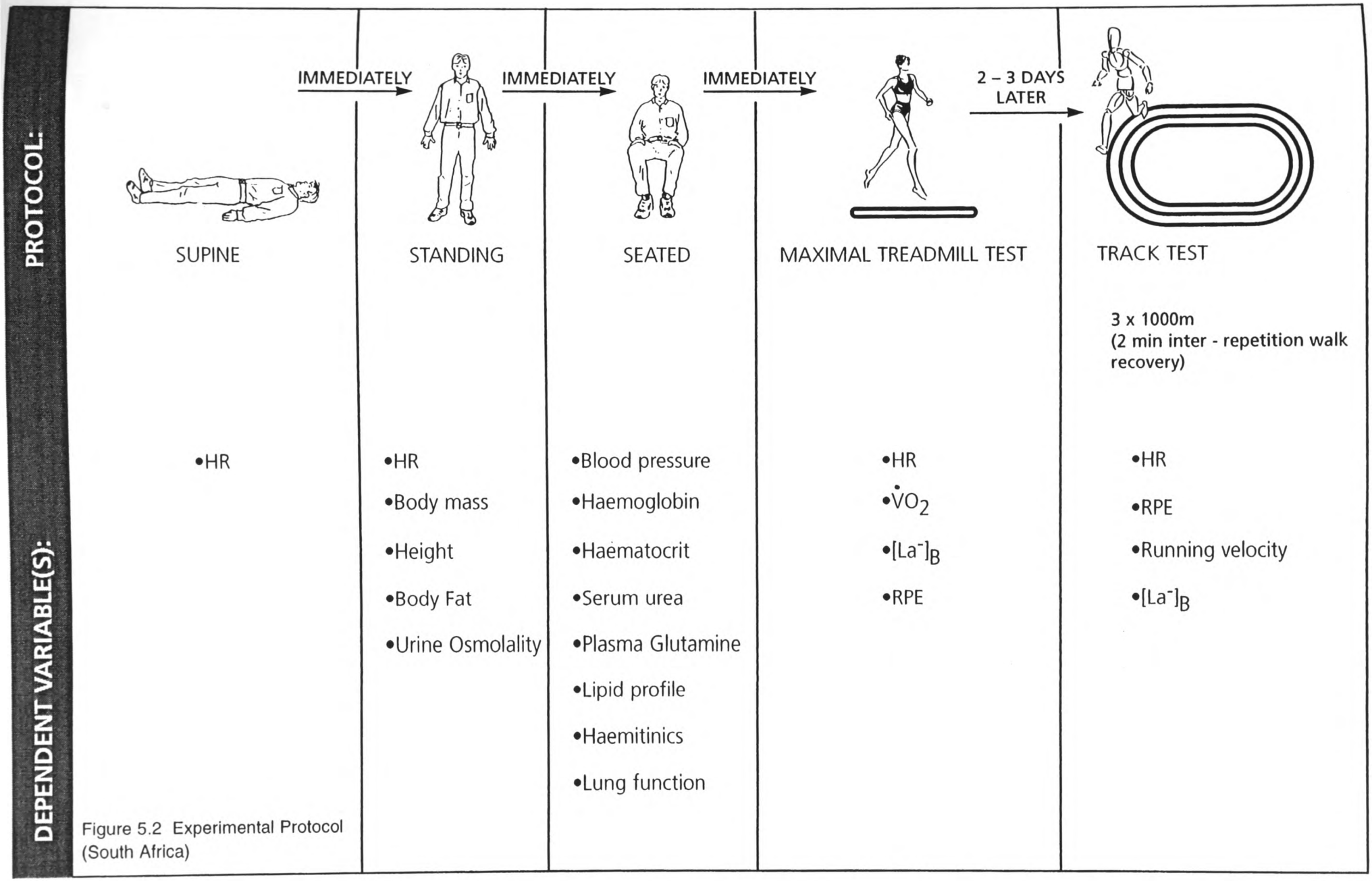


Figure 5.2 Experimental Protocol (South Africa)

5.2.3.1 Pre-Altitude Testing (PRE)

Pre-altitude laboratory and field based physiological tests were conducted at sea-level approximately 3 weeks prior to the start of an altitude training camp which was based at Krugersdorp, S.Africa (~1,640 m).

5.2.3.1.1 Resting Measurements

Each subject was instructed to refrain from any physical exercise for 48 h prior to reporting to the laboratory following a 12 h overnight fast. A venous blood sample was collected by antecubital venepuncture between 07:00 h to 10:00 h. Samples were subsequently analysed for a full blood count, blood lipids including apolipoproteins A and B and lipoprotein (a), plasma glutamine, serum ferritin, serum vitamin B_{12}, red cell folate, plasma iron and transferrin. Detailed analyses of the analytical and methodological procedures involved are discussed in Chapter 3. Each subject also completed a questionnaire which was designed to determine the onset, duration and physical symptoms of any respiratory or gastrointestinal infections (Appendix M).

5.2.3.1.2 Exercise Measurements

5.2.3.1.2.1 Laboratory Test

A minimum of 2 treadmill sessions were performed prior to study 2 to control for the contaminating effects of habituation (Appendix K). Each subject performed a 4 minute warm-up on a treadmill at a velocity that elicited 60% of age predicted maximum heart rate (APMHR) determined as:

$$\textit{APMHR (b.min}^{-1}\textit{)} = \textit{220 (b.min}^{-1}\textit{)} - \textit{Age (Years) Robinson (1938)}$$

Following a warm-up consisting of 5 minutes of flexibility and calisthenics, each subject performed a continuous incremental treadmill test designed to determine $\dot{V}O_{2max}$ according to the protocol of Taylor et al. (1955). The starting treadmill velocity was set at 4.42 $m.s^{-1}$ for male subjects and 3.81 ms^{-1} for female subjects with the treadmill grade at 3.5%. Whilst velocity was maintained constant throughout the test, the treadmill grade was increased by 2.5% in 3 minutes increments. Borg ratings of perceived exertion [(RPE), Borg, 1973] and heart rate (HR) were recorded between 2:30 to 3:00 minutes of each incremental stage. The subject raised his/her left hand when they felt that they could

continue for only 1 more minute before volitional exhaustion during which time expired gas was collected into a series of 150 L Douglas Bags. These were subsequently analysed for $\dot{V}_E$, $\dot{V}O_2$, $\dot{V}CO_2$, and RER. A detailed account of the analytical procedures involved during the manual measurement of respiratory gas exchange parameters is discussed in Section 3.9.1.3. Running time to exhaustion, maximal HR and maximal whole blood lactate ($[La^-]_B$) were also determined. To ensure that $\dot{V}O_{2max}$ was attained, the following criteria had to be satisfied: [1 Attainment of a peak HR within 5% of the MAHR and [2 An RER of > 1.1. On completion of the $\dot{V}O_{2max}$ test, each subject performed a walk recovery at 1.39 $m.s^{-1}$ for 3 minutes. HR and $[La^-]_B$ were determined at the end of the recovery period.

5.2.3.1.2.2 Track Test

An identical track session to that conducted in the previous study was performed by each subject on a tartan track at sea-level. The only variation being three as opposed to four repetitions of 1000 m were performed separated by a 2 minute recovery walk. This decision was based on feedback from the subjects who had participated in Study 1 who felt that their previous track session at altitude had been too physically demanding.

5.2.3.1.2.3 Training Load

Total weekly running distances and HR's were recorded one week prior to PRE testing. Each subject was issued with an ECG calibrated short-range telemetry system (Polar Vantage NV™, Polar Electro Oy, Finland) to record the measurement of exercise HR. Training distances were divided into track (HR~170-185 $b.min^{-1}$) and steady state (HR~140-160 $b.min^{-1}$) running sessions. All subjects were instructed to continue with their current training programme throughout the duration of the study.

5.2.3.2 Altitude Testing (ALT)

5.2.3.2.1 Resting Measurements

Physiological and medical equipment was flown from London, UK to Johannesburg, S.Africa and subsequently transported to a laboratory based at Krugersdorp (~1,640 m). Physiological testing was organised in collaboration with Dr. R Carter (Director of the Exercise Science Laboratory, Witwatersrand Medical School, Johannesburg, S.Africa).

All resting physiological measurements were conducted immediately on waking between 0600 h and 1100 h on days 15 to 27 at altitude following a 12 h overnight fast. Dependent variables measured included; blood pressure, heart rate [(HR) supine and standing], body mass, haemoglobin (Hb), packed cell volume (PCV) , serum urea and urine osmolality. Lung function was assessed on day 16 at altitude. A resting venous blood sample was obtained from each subject on day 19 to 20 at altitude and immediately stored at -70°C. These samples were transported back to the UK in liquid nitrogen and subsequently analysed for plasma glutamine concentration.

5.2.3.2.2 Exercise Measurements

5.2.3.2.2.1 Laboratory Test

An identical treadmill protocol to that performed at sea-level was repeated in a temperature controlled (21 ± 1°C) laboratory based at Witwatersrand Medical School, Johannesburg, S.Africa (1,640 m). Ventilatory and pulmonary gas exchange parameters were determined on-line using a MedGraphics^{R} Cardiopulmonary exercise systems CPX/D (Cardiokinetics, UK). This respiratory model had been previously validated by comparing respiratory data measured during a standardised $\dot{V}O_{2max}$ test with a manual system (Appendix I).

Arterial oxygen saturation (SaO_2) measurements were also displayed continuously throughout the test with an ear oximeter (Biox 3000, Ohmeda). Data were recorded during the last 30 s of each incremental stage and at the point of physical exhaustion.

5.2.3.2.2.2 Track Test

An identical track session to that performed at sea-level was repeated on a tartan track between weeks 3 to 4 at Krugersdorp, S.Africa (1,640 m). The National Endurance Coach advised each subject to perform the session as consistently as possible.

5.2.3.2.2.3 Training Load

The EXP group was instructed to perform all altitude training sessions at the same relative exercise intensity as that conducted at sea-level (i.e. same HR). In comparison to the New Mexico study, the altitude terrain in the present study was less mountainous and there were fewer grass fields. As a consequence of this, the majority of training had to be conducted on concrete roads. Total weekly running distance and intensity were recorded during the altitude sojourn.

5.2.3.3 Post-Altitude Testing (POST)

Identical resting and exercise measurements were repeated 10 and 20 days following the EXP group's return to sea-level.

Data were analysed using parametric and non-parametric statistics. A detailed account of the specific analyses employed and the mathematical rationale are discussed in Chapter 3.

5.3 RESULTS AND DISCUSSION

5.3.1 Environmental Conditions

Figure 5.3 illustrates the changes in barometric pressure and ambient temperature during the experimental period. Barometric pressure averaged 768 mmHg at sea-level (Range: 766 mmHg - 769 mmHg) and decreased to a mean of 630 ± 1 mmHg at 1,640 m (Range: 629 mmHg - 632 mmHg). The decrease in barometric pressure at altitude represented a 29 mmHg decrease (-19%) in P_IO_2 (151 mmHg to 122 ± 1 mmHg).

Changes in ambient temperature have profound implications for physiological function (Young, 1988; Sutton, 1994). However, the changes encountered in the present study are unlikely to have had any effect on metabolic or respiratory control. Sea-level ambient temperature averaged 14.6 °C at sea-level (Range: 11.4 - 19.6C) and increased to a mean value of 18.5 ± 3.3°C at altitude (Range: 16°C - 28°C).

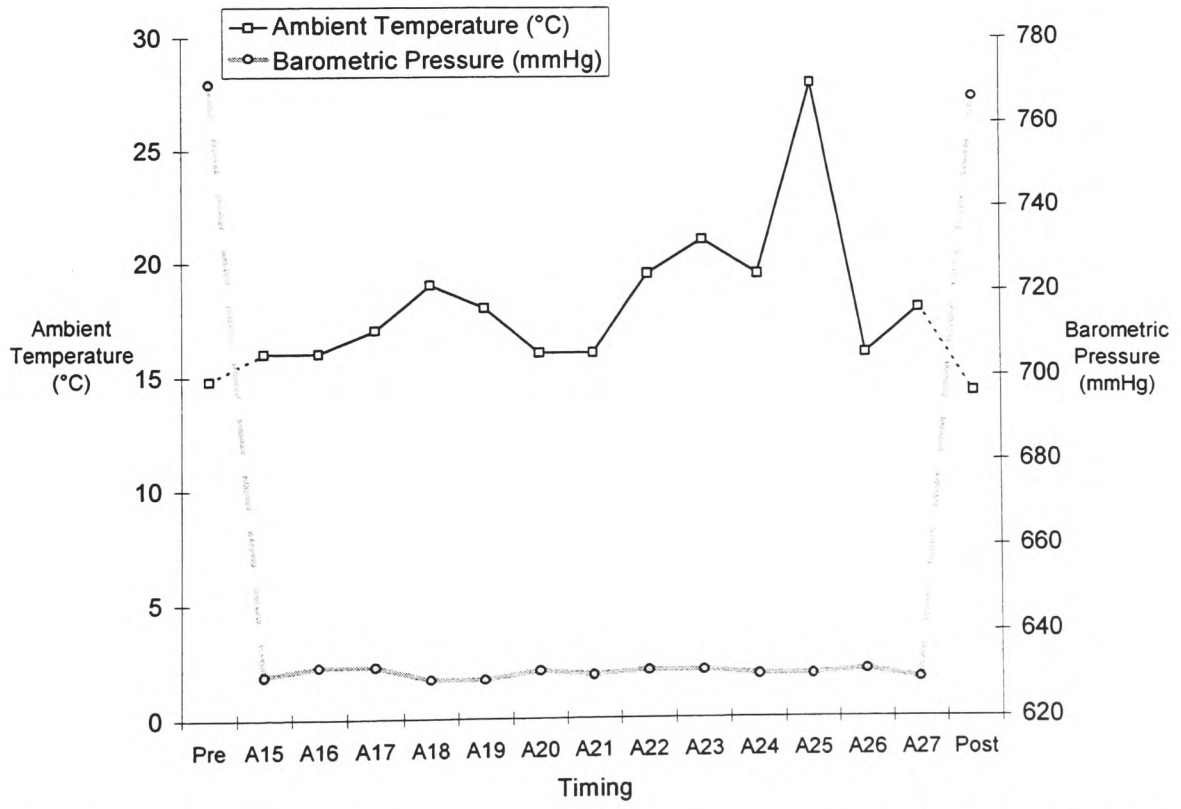


Figure 5.3 Ambient Temperature and Barometric Pressure at Sea-Level and Altitude

Pre: Pre-altitude
A15 - A27: Days 15 to 27 at altitude (1,640 m)
Post: 20 days post-altitude

5.3.2 Training Load

Two males and the only female subject from the EXP group were diagnosed with lower limb stress fractures during the 3rd week at altitude and were thus excluded from the overall analyses (EXP n = 7). It has been suggested that a decrease in the fluoride content of drinking water during several weeks at altitude may be implicated in a decrease in bone density and thus increase the risk of musculoskeletal injury (personal communication, Professor C.Sharpe, Brunel University, UK). However, the stress fractures were most likely the consequence of repetitive high impact on concrete surfaces due to the lack of softer running surfaces.

Figure 5.4 and Table 5.4 summarise the changes in weekly training volume and intensity during the experiment. There were no significant differences between PRE EXP and PRE CON group mean steady state, (HR~140-160 $b.min^{-1}$), track (HR~170-185 $b.min^{-1}$) and total weekly running distances. $POST_2$ (20 days following return to sea-level) EXP group mean steady state and total weekly running distances were significantly lower than the PRE EXP group mean values ($P < 0.05$). EXP track running distances did not change during the experiment. CON group total weekly running distance was significantly lower by the $POST_2$ test ($P < 0.05$) due to the significant decrease in track running distance ($P < 0.01$). The reductions in EXP and CON total weekly training distance during $POST_2$ testing occurred because subjects tapered in preparation for a major cross country race, 4 days prior to physiological testing.

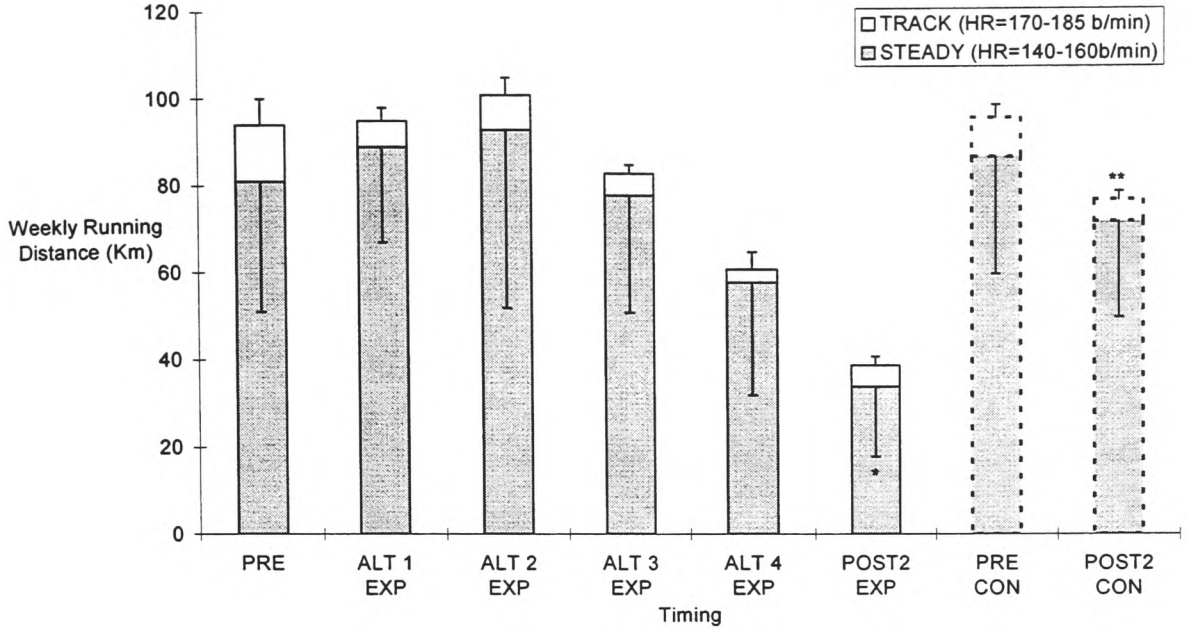


Figure 5.4 Weekly Training Load at Sea-Level and Altitude (n = 19)
Values are Mean ± SD
*: Significantly different from within group PRE value ($P < 0.05$)
**: Significantly different from within group PRE value ($P < 0.01$)
PRE: Pre-altitude
ALT1-4: Weeks 1 to 4 at 1,640 m
$POST_2$: 20 days post-altitude
EXP: Altitude group (n) = 7
CON: Sea-level group (n) = 12

Table 5.4 Total Weekly Running Distance at Sea-Level and Altitude (n = 19)

Timing	Group	Weekly Distance (km)	*Range (km)*
PRE	EXP	95 ± 34	*66 - 140*
ALT 1	EXP	95 ± 24	*81 - 130*
ALT 2	EXP	101 ± 45	*63 - 166*
ALT 3	EXP	83 ± 28	*61 - 124*
ALT 4	EXP	61 ± 29	*35 - 103*
$POST_2$	EXP	39 ± 16†	*24 - 53*
PRE	CON	97 ± 27	*68 - 159*
$POST_2$	CON	77 ± 23†	*21 - 114*

Values are Mean ± SD *(Range)*
†: Significantly different from within group PRE value ($P < 0.05$)
PRE: Pre-altitude
ALT1-4: Weeks 1 to 4 at 1,640 m
$POST_2$: 20 days post-altitude
EXP: Altitude group (n) = 7
CON: Sea-level group (n) = 12

A summary of the type of training sessions and weekly running distances covered on consecutive days at 1,640 m (A15 - A27) is presented in Figure 5.5 and Table 5.5.

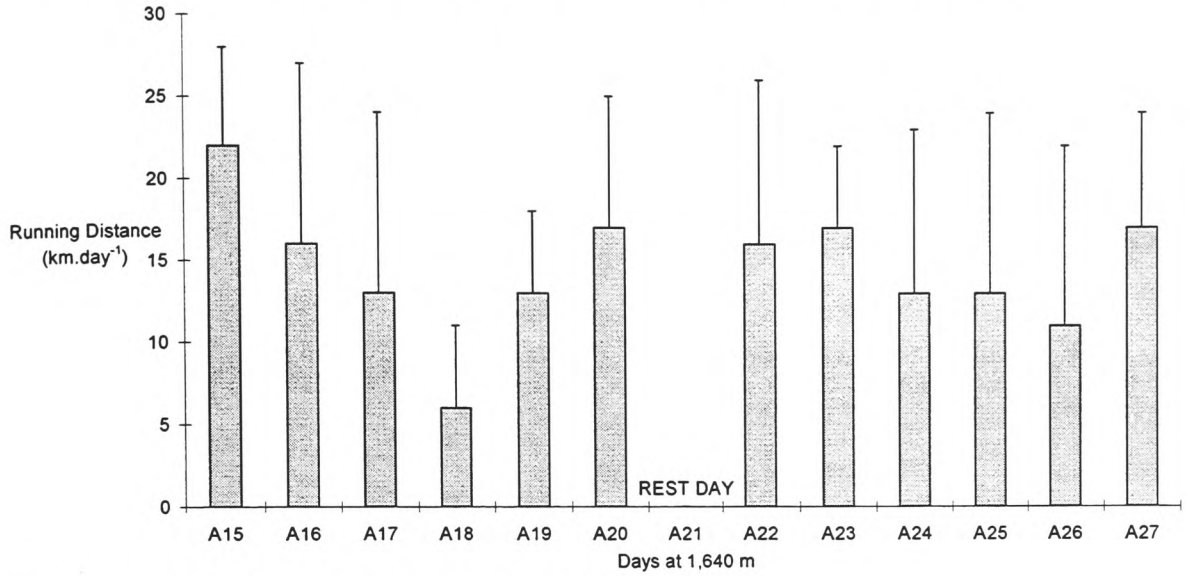


Figure 5.5 Daily Running Distances at Altitude (n = 7)
Values are Mean ± SD
A15 - A27: Days at 1,640 m

Table 5.5 Training Sessions Conducted at Altitude (~1,640 m)

Day at Altitude	Session (pm)*
15	Hill sprints on 30° incline (10 x 150 m followed by 6 x 100m)
16	Long steady road run
17	Steady road run
18	Track session (Either 3 x 1000 m or 5 x 2000 m)
19	Laboratory $\dot{V}O_2$max treadmill test
20	Steady road run
21	Rest or steady road run
22	Track session (3 x 1000 m)
23	Long steady road run
24	Steady road run
25	Track session (8 - 16 x 400 m)
26	Steady road run
27	Steady road run

* - All pm sessions were preceded by an early morning 6-8 km easy road run (HR~140-160 b.min^{-1})
EXP (n) = 7

5.3.3 Resting Adaptations

A summary of the changes in selected physiological parameters measured at rest during the study is presented in Tables 5.6 to 5.18.

5.3.3.1 Anthropometric Adaptations

Sixty nine days of sea-level training or an identical period of combined sea-level and altitude training resulted in significant changes in body composition. Table 5.6 demonstrates that whilst body mass did not change during the study, EXP group mean sum of skinfolds and estimated body fat were significantly greater at altitude and following return to sea-level ($P < 0.01$ vs pre-altitude). CON group mean sum of skinfolds and estimated body fat were also significantly greater during $POST_2$ testing ($P < 0.01$ vs pre-altitude). The magnitude of increase in sum of skinfolds ($POST_2$ - PRE) was comparable between groups (EXP: +15% vs CON: +17%) and was considered physiological significant, assuming a standard error of 5%, (Durnin and Womersley, 1974 and Lohman, 1981).

Table 5.6 Anthropometric Adaptations at Sea-Level and Altitude (n = 19)

Variable	Body Mass (Kgs)		Sum of Skinfolds mm		Body Fat (%)	
Group	**EXP**	**CON**	**EXP**	**CON**	**EXP**	**CON**
PRE	69.6 ± 8.3	64.4 ± 7.2	23.2 ± 4.0	23.5 ± 6.1	8.5 ± 2.0	11.3 ± 5.2
ALT	69.6 ± 8.2	.....	26.0 ±3.8‡	.....	10.1 ± 1.7‡	
$POST_1$	70.4 ± 8.1	.....	26.4 ±3.9‡	.....	10.2 ± 1.7‡	
$POST_2$	70.1 ± 8.3	64.4 ± 7.0	26.6±4.0‡	27.4±9.9‡	10.2 ± 2.0‡	12.9 ± 6.1‡

Values are Mean ± SD
‡: Significantly different from within group PRE value ($P < 0.01$)
PRE: Pre-altitude
ALT: Values represent mean values obtained between days 15 to 27 at 1,640 m
$POST_1$ / $POST_2$: Determined 10 and 20 days following EXP group return to sea-level
EXP: Altitude group (n = 7)
CON: Sea-level group (n = 12)

These data would suggest that EXP and CON group mean values for fat free mass (FFM) decreased ($P < 0.01$) between PRE and $POST_2$ tests (Figure 5.6). The decrease in the EXP group FFM was evident by the 3rd week at 1,640 m.

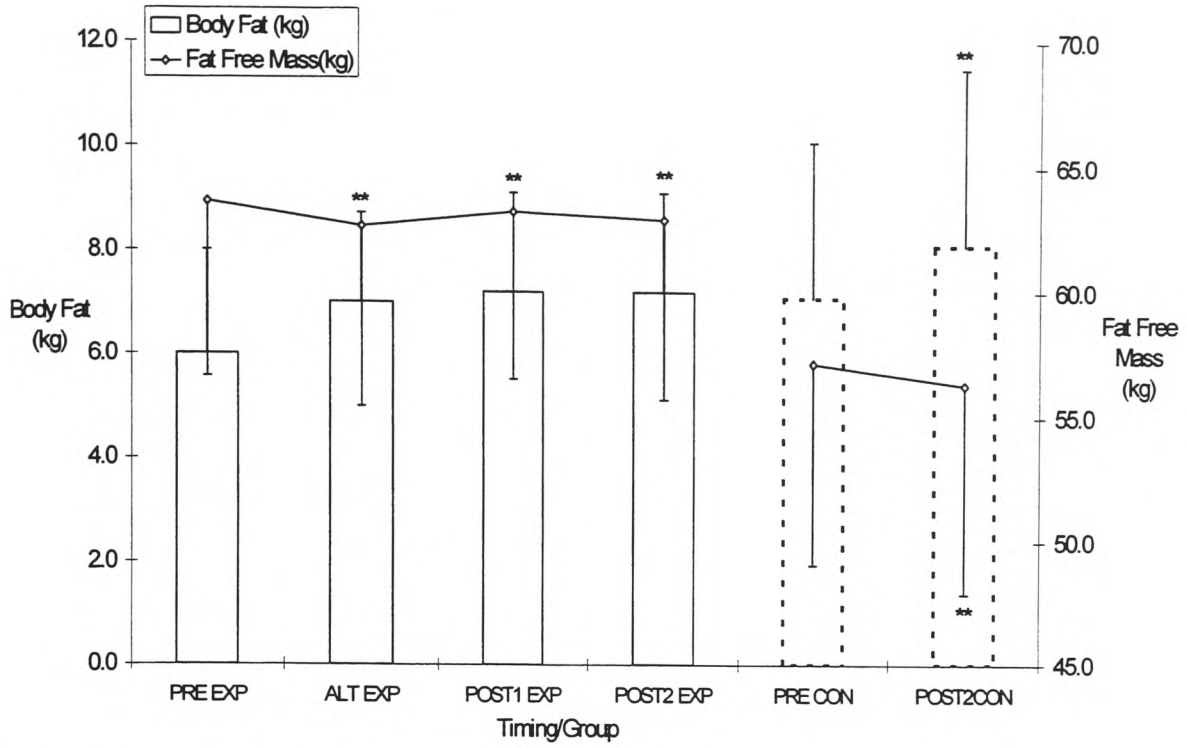


Figure 5.6 Body Composition at Sea-Level and Altitude
Values are Mean ± SD

**:	Significantly different from within group PRE value ($P < 0.01$)
PRE:	Pre-altitude
ALT:	Mean value at 1,640 m
POST1:	10 days post-altitude
POST2:	20 days post-altitude
EXP:	Altitude group (n) = 7
CON:	Sea-level group (n) = 13

These data would suggest that the changes in body composition observed in the EXP group following return to sea-level were not caused by hypoxia *per se* as identical changes were also noted in the normoxically-trained CON group. It is conceivable that an increased intake of dietary fats and/or a decrease in energy expenditure (Figure 5.4 and Table 5.4) would account for these anthropometric changes. Control of nutrient intake is considered an integral part of a well designed study to isolate the independent effects of hypoxia at altitude (Houston et al 1987 and Rose et al 1988). However, dietary intake was not controlled in the present study due to financial constraints and thus should be considered as a potentially confounding variable.

5.3.3.2 Hydration Status

Hydration status is most accurately quantified using stable isotope methodology which is often impractical due to the prohibitive costs and invasive nature of measurement (Schoeller et al 1984). Thus, the present study incorporated the measurement of urine

osmolality which has been shown to be a valid determinant of hydration status (Costill and Sparks, 1973 and Baker et al 1983).

Previous investigators have reported increases in serum and urine osmolality after 14 to 27 days at significantly greater altitudes ranging between 5245 m to 6,300 m (Blume et al 1984 and Hackney et al 1995). However, there were no changes observed in urine osmolality in the present study either at sea-level or at altitude (Table 5.7 and Figure 5.7). Thus, it would appear that hypoxia per se did not result in any significant losses in total body water content. Assuming that a urine osmolality of between 200 mosmol.kg^{-1} to 600 mosmol/kg represents euhydration (Armstrong, 1994), it would appear that the EXP group were hypohydrated 10 days following return to sea-level. The CON group also appeared to be hypohydrated throughout the duration of the study. Using the same analytical methods, (freezing point depression), urine osmolalities of 1295 ± 75 mosmol.kg^{-1} and 1056 ± 206 mosmol.kg^{-1} have been measured in International male and female Judo players respectively in Tallahassee, USA during preparation for the US Open Championships (Pollock et al 1996). In that same study, a female subject was recorded with a urine osmolality of 1361 mosmol.kg^{-1}, a value which has not been previously documented in the literature (personal communication, Professor R.Maughan, University of Aberdeen, UK). The highest individual value recorded in the present study was 1086 mosmol.kg^{-1} in a male subject from the CON group.

Table 5.7 Urine Osmolality (mosmol.kg^{-1}) at Sea-Level and Altitude

Group (n)	PRE	ALT	$POST_1$	$POST_2$
EXP (7)	386 ± 193	598 ± 151	746 ± 274	580 ± 316
Range:	*197 - 670*	*441 - 794*	*329 - 1017*	*243 - 1060*
CON (13)	761 ± 332	.....	.....	614 ± 267
Range:	*184 - 1086*	.....	.....	*192 - 1050*

Values are Mean ± SD and *Range*
PRE: Pre-altitude
ALT: Values represent mean values obtained between days 15 to 27 at 1,640 m
$POST_1$ / $POST_2$: Determined 10 and 20 days following EXP group return to sea-level
EXP: Altitude group
CON: Sea-level group

Subjects in the EXP group were instructed to increase their fluid intake during the altitude sojourn if urine osmolalities exceeded 600 mosmol.kg^{-1}, a threshold concentration which has been associated with moderate hypohydration (Armstrong, 1994 and personal communication, Professor B.Davies, University of Glamorgan, UK). Despite these recommendations, EXP group mean urine osmolalities exceeded this threshold value on days 15, 17, 18, 22, 24 and 27 at altitude (Figure 5.7).

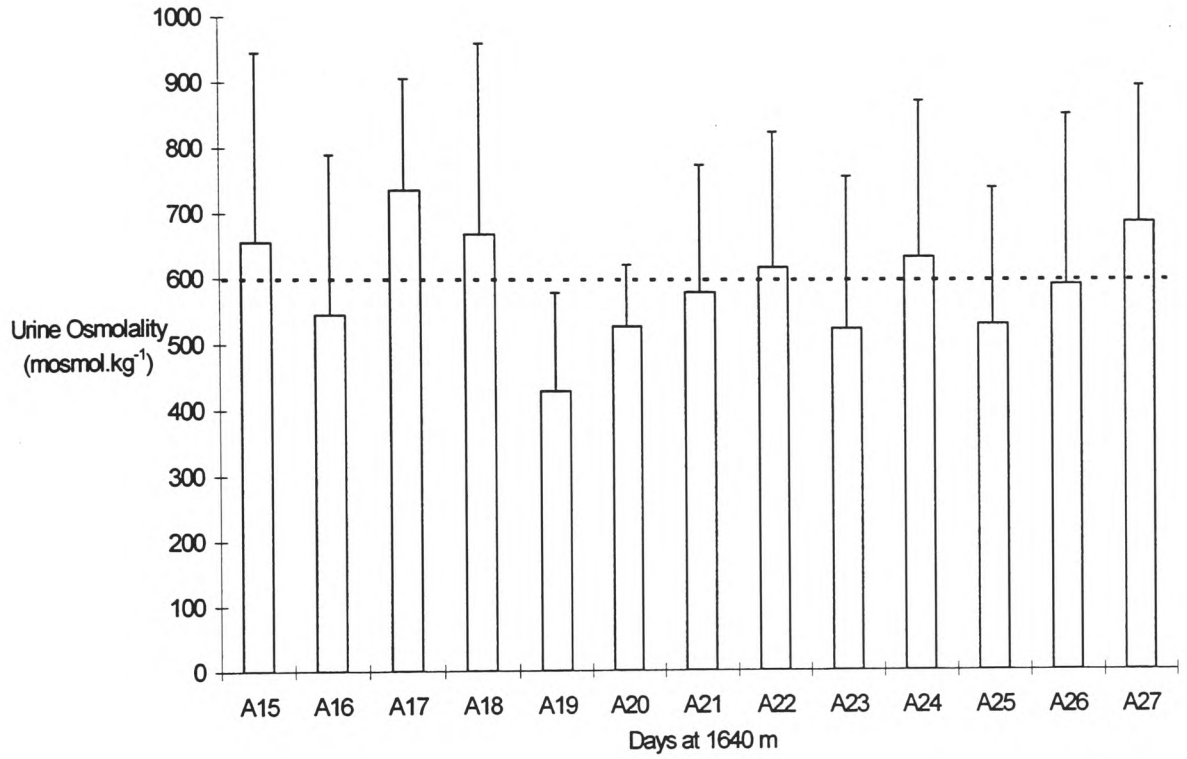


Figure 5.7 EXP Group Urine Osmolality on Consecutive Days at 1,640 m (n = 7)
Values are Mean ± SD
Emboldened line represents group mean value at altitude

5.3.3.3 Haematological Adaptations

5.3.3.3.1 Iron Status

Iron is the most important "erythropoiesis specific" nutritional factor (Berglund, 1992) and it is therefore critical to ensure complete haematological adaptation to environmental hypoxia. Iron is absorbed in the small intestine where it combines with a transport protein, transferrin (Clarkson, 1990). Transferrin transports iron to the bone marrow where it is captured by transferrin receptors on the surface of proliferating erythroid normoblasts (Magnusson et al 1984 and Huebers and Finch, 1987). Excess iron is stored within ferritin and haemosiderin in cells of the reticuloendothelial system, hepatocytes and erythroid

precursor in the bone marrow (Diess, 1982). A detailed account of the kinetics of iron metabolism has recently been reviewed by Smith (1995).

There is evidence which suggests that iron demand and mobilisation are increased at altitude to support the elevated reticulocytosis (Reynafarje et al 1959, 1961; Klausen et al 1991; Roberts et al 1992 and Stray-Gundersen et al 1994). During the first days of exposure to 4,500 m in sea-level natives the absorption of iron was 3.8 times higher, the clearance of iron from the plasma had increased by 35% and the iron uptake for erythrocyte formation had increased by 100% (Reynafarje et al 1959, 1961). A significant decrease in iron saturation of transferrin ($P < 0.05$ vs pre-altitude value) was recorded in 6 elite cross country skiers following return to sea-level after a 7 day sojourn to an altitude of approximately 1,695 m to 2,700 m (Klausen et al 1991). This represented manifest iron deficiency or iron deficient erythropoiesis (15% saturation) 4 days following return to sea-level (Klausen et al 1991). As previously discussed in Section 2.8.5, a high prevalence of a typical iron deficiency has been observed in distance runners even *at sea-level* due to an increased gastrointestinal blood loss of iron (Nachtigall et al 1996). Thus, a combination of intense physical exercise *and* environmental hypoxia could further increase the disparity between iron supply and demand and subsequently suppress the synthesis of Hb at altitude. Indirect evidence for this has been provided by Hannon et al. (1967, 1969) and more recently by Stray-Gundersen et al. (1992). Hannon et al. (1967, 1969) demonstrated that in comparison to a control group (untreated subjects), women who received oral iron supplements (200 to 300 mg of oral iron per day) for 3 months at sea-level experienced greater increases in Hb and packed cell volume during a 10 week exposure to 4,300 m. However, the risk of developing bowel cancer needs to be considered before iron supplementation can be advocated.

However, there was no evidence that hypoxia per se increased iron demand and mobilisation in the present study (Table 5.8). There were no significant correlations between the changes in Hb (POST - PRE value) and the changes (POST - PRE value) in concentrations of serum iron, transferrin and serum ferritin for both EXP and CON groups. All physiological indices of iron status remained stable throughout the study in the EXP group. However, a significant decrease ($P < 0.05$ vs PRE value) in serum vitamin B_{12} and red cell folate was observed by the $POST_2$ test in the CON group only. The stable reticulocyte counts in the CON group (Table 5.9) would suggest that these changes were

not associated with increased erythropoietic activity and were most likely the consequence of a decreased dietary intake of B-complex vitamins.

Table 5.8 Subject Iron Status at Sea-Level (n = 17)

Variable	Reference	PRE EXP	$POST_2$ EXP	PRE CON	$POST_2$ CON
Serum Ferritin	**20 - 300 (♂)**	48 ± 35	46 ± 20	42 ± 33	44 ± 27
(ng/ml)	**10 - 300 (♀)**	*(19 - 110)*	*(19 - 85)*	*(11 - 110)*	*(11 - 99)*
Serum Iron	**9.0 - 29.0**	22.8 ± 3.7	24.5 ± 4.8	26.4 ±11.0	21.7 ± 7.7
(µmol/L)	(♂ / ♀)	*(17.8 - 27.3)*	*(18.4 - 32.1)*	*(5.1 - 44.0)*	*(7.3 - 29.6)*
Transferrin	**1.7 - 3.4**	2.9 ± 0.3	2.9 ± 0.2	3.3 ± 1.7	2.7 ± 0.4
(g/L)	(♂ / ♀)	*(2.0 - 3.3)*	*(2.6 - 3.1)*	*(2.1 - 8.1)*	*(1.7 - 3.3)*
Vitamin B_{12}	**180 - 1132**	574 ± 171	495 ± 20	956 ± 56	845 ± 126†
(pg/ml)	(♂ / ♀)	*(433 - 933)*	*(460 - 529)*	*(859 - 1070)*	*(551 - 1020)*
Red Cell	**125 - 600**	436 ± 117	372 ± 22	491 ± 112	343 ± 101†
Folate (ug/L)	(♂ / ♀)	*(291 - 649)*	*(327 - 392)*	*(220 - 627)*	*(234 - 551)*

Values are Mean ± SD and *(Range)*

†: Significantly different from within group PRE value ($P < 0.05$)

PRE: Pre-altitude

$POST_2$: Determined 20 days following EXP group return to sea-level

EXP: Altitude group (n = 7)

CON: Sea-level group (n = 10)

The depressed serum ferritin concentrations observed in the present study were similar to values ranging between 30 to 60 ng/ml that have been previously documented in distance runners (Magnusson et al 1984; Resina et al 1988; Weight et al 1992 and Nachtigall et al 1996). According to Nachtigall et al. (1996) who quantified iron balance in elite distance runners using sophisticated radio-labeling techniques, a serum ferritin concentration of < 35 ng/ml would represent partly depleted iron stores and < 12 ng/ml would represent totally depleted iron stores. Using these critical values, individual data from the present study suggested that 4 subjects from the EXP group (57%) and 6 subjects in the CON group (60%) were iron deficient during PRE testing. This was noted despite widespread oral iron supplementation equivalent to 200 mg per day of ferrous sulphate. The lowest concentration of serum ferritin recorded in the EXP group was 19 ng/ml for a male subject

and 11 ng/ml was recorded in a male and female subject in the CON group. It was not possible to differentiate between latent (advanced form of iron deficiency) as opposed to prelatent iron deficiency (depleted iron stores) in the present study. However, in a cohort of elite distance runners with similar concentrations of serum ferritin (mean value of 35 ± 12 ng/ml), Nachtigall et al. (1996) demonstrated that ^{59}Fe mucosa plasma transfer and ^{59}Fe incorporation rate into erythrocytes was normal, suggestive of *iron store depletion*.

5.3.3.3.2 Haemoglobin Concentration (Hb) and Packed Cell Volume (PCV)

Table 5.9 summarises the changes in venous Hb, PCV and reticulocyte count at sea-level and altitude. Whilst there was a significant correlation ($r = 0.96$, $P < 0.001$) between the increase in EXP group Hb (POST - PRE value) and reticulocyte count (POST - PRE value), the tendency for an increased EXP group mean resting Hb concentration and reticulocyte count at altitude and following return to sea-level did not attain statistical significance. As a consequence, resting arterial oxygen content (CaO_2) did not change during the study (Table 5.10). However, EXP group mean PCV increased significantly ($P < 0.05$ vs PRE) following 20 days return to sea-level and the data presented in Table 5.7 would suggest that this was independent of a haemoconcentration and most probably the consequence of an erythropoietic response to environmental hypoxia.

Table 5.9 Selected Haematological Parameters at Sea-Level and Altitude (n = 17)

Variable	PRE EXP	ALT EXP*	POST$_2$ EXP	PRE CON	POST$_2$ CON
Hb	14.7 ± 0.9	15.2 ± 1.1	15.5 ± 0.5	15.0 ± 0.9	15.0 ± 1.2
(g/dl)	*(13.2 - 16.0)*	*(13.7 - 16.6)*	*(14.6 - 16.0)*	*(13.7 - 16.1)*	*(13.1 - 17.0)*
PCV	0.47 ± 0.04	0.45 ± 0.04	0.51 ± 0.01†	0.48 ± 0.03	0.49 ± 0.03
(L/L)	*(0.40 - 0.51)*	*(0.40 - 0.51)*	*(0.49 - 0.52)*	*(0.44 - 0.52)*	*(0.43 - 0.54)*
Ret. count	4.88 ± 0.44	5.02 ± 0.54	5.09 ± 0.20	4.97 ± 0.25	4.83 ± 0.34
($x10^{12}$/L)	*(4.06 - 5.23)*	*(4.30 - 5.64)*	*(4.72 - 5.38)*	*(4.55 - 5.26)*	*(4.25 - 5.43)*

Values are Mean ± SD and *(Range)*

†: Significantly different from within group PRE value ($P < 0.05$)

Hb: Haemoglobin

PCV: Packed cell volume

Ret.count: Reticulocyte count

PRE: Pre-altitude

ALT*: Determined during days 19 to 20 at 1,640 m

POST$_2$: Determined 20 days following EXP group return to sea-level

EXP: Altitude group (n = 7)

CON: Sea-level group (n = 10)

Table 5.10 Estimation of Resting Arterial Oxygen Content (CaO_2) at Sea-level and Altitude (n = 17)

Timing/ Group	Hb (g/dl)	SaO_2 (%)*	PaO_2 (mmHg)#	O_2 bound to Hb (mlO_2/dl)	Dissolved O_2 (ml/dl)	CaO_2 mlO_2/dl
PRE EXP	14.7	97.4	91	19.13 ± 1.18	0.27 ± 0.00	19.40 ± 1.18
ALT EXP*	15.2	93	83	19.00 ± 1.43	0.25 ± 0.00‡	19.25 ± 1.43
$POST_2$ EXP	15.5	97.4	91	20.23 ± 0.59	0.27 ± 0.00	20.50 ± 0.59
PRE CON	15.0	97.4	91	19.51 ± 1.21	0.27 ± 0.00	19.78 ± 1.21
$POST_2$ CON	15.0	97.4	91	19.63 ± 1.56	0.27 ± 0.00	19.90 ± 1.56

Values are Mean ± SD

‡: Significantly different from within group PRE value ($P < 0.01$)
*: derived from Tucker et al (1984)
#: derived from Dempsey et al (1984)
PRE: Pre-altitude
ALT*: Determined during days 19 to 20 at 1,640 m
$POST_2$: Determined 20 days following EXP group return to sea-level
EXP: Altitude group (n = 7)
CON: Sea-level group (n = 10)

Unfortunately, due to the batch analysis of blood samples, the results of both haematinic assays and full blood counts were not available until 20 days following the EXP group's return to sea-level ($POST_2$). Thus, the 4 subjects in the EXP group who were iron deficient (serum ferritin < 35 ng/ml) prior to the altitude sojourn did not receive further iron supplementation until the conclusion of the study. Medical treatment was administered by the Olympic Medical Officer, British Olympic Medical Centre, UK. It is therefore conceivable that their already depleted iron stores could not meet the demand for Hb synthesis at altitude (Table 5.9). As previously discussed, it would seem likely that the haematological response to altitude training may have been even more pronounced assuming that iron stores were optimal at sea-level.

Whilst it was not possible to obtain repeated venous blood samples on consecutive days at altitude, resting concentrations of Hb and PCV in *arterialised capillary blood* (earlobe sample) were determined using portable equipment. A detailed account of the analytical procedures involved is discussed in Sections 3.6.2.1 and 3.6.2.2. Significant correlations were observed between pooled EXP and CON group resting concentrations of Hb and PCV determined from venous and arterialised capillary blood samples during PRE (Hb r value =

0.51, $P < 0.05$ and PCV r value = 0.65, $P < 0.05$) and $POST_2$ testing (Hb r value = 0.77, $P < 0.01$ and PCV r value = 0.66, $P < 0.05$). There were no significant differences between measurement site and metabolite concentration which would suggest that the portable techniques proved to be both an *accurate* and *precise* means of determining haematological status. However, in comparison to PRE EXP group mean values, there were no significant changes in either resting Hb or PCV during days 15 to 27 at 1,640 m or following 10 days return to sea-level ($POST_1$).

5.3.3.3.3 Plasma Glutamine and Immune Function

An increased frequency of upper respiratory tract infections (URTI) and diarrhoea was observed during the altitude sojourn in the previous study and two male subjects subsequently contracted infectious mononucleosis. An increased incidence of URTI has previously been reported during altitude training camps (personal communication, Mr G.Gandy, National Endurance Coach for 5 km to 10 km) yet there is no scientific evidence to support this observation. These observations suggest an association between hypoxia and adverse changes in immune function. Epidemiological data tend to support this hypothesis, yet the physiological impact of multiple stressors encountered at altitude such as cold, dehydration, novel infectious agents and poor hygiene cannot be excluded (Meehan et al 1988). Higher infant mortality due to respiratory infections has been reported among highland natives (Chohan et al 1975) and an increased prevalence of pneumonia has been identified among military troops based at high altitudes (Singh et al 1977). Soviet scientists have also reported an increased frequency of respiratory infections among natives residing higher than 2,600 m (Kitaev and Tokhtabayev, 1981). It is also interesting to speculate that the release of vasoactive inflammatory mediators during a subclinical infection at altitude may contribute to oedema. This hypothesis awaits investigation.

A limited number of prospective studies have investigated the immunomodulatory effects of hypoxia on human immunobiology. As part of Operation Everest II, Meehan et al. (1988) investigated the effects of progressive decompression over 28 days to equivalent altitudes of 2,286 m and 7,620 m on *in vitro* and *in vivo* immunologic function in 7 male subjects. In comparison to sea-level measurements, the authors demonstrated significant reductions in mitogen-stimulated mononuclear-cell activation and proliferation and a tendency towards a decreased phytohemagglutinin-stimulated interferon production at altitude. Whilst these results provided *in vitro* evidence of hypoxia-mediated depression of T-cell activation, B-

cell function and mucosal immunity remained unaltered. The authors failed to elucidate the physiological mechanisms responsible for the decreased *in vitro* cellular responses to mitogenic stimulation. However, the contributory immunomodulatory roles of endogenous glucocorticoids and neuropeptides which are increased at altitude (Richalet et al 1989; Anand et al 1993 and Rusko et al 1996) may contribute to the observed alterations in mononuclear-cell function and subsequent immune competence.

In light of these observations, immunocompetence was indirectly determined in the present study by quantifying the effects of chronic altitude exposure on glutamine metabolism.

Newsholme and Leech (1983) first demonstrated that the transamination of BCAA by *skeletal muscle* provided nitrogen for the subsequent formation of glutamine. The role of glutamine release from skeletal muscle in the control of immune function has been the topic of much interest over the last decade and has most recently been reviewed by Rowbottom et al (1996).

Ardawi and Newsholme (1983) reported that, in addition to glucose, glutamine was as an important substrate for key cells of the immune system, in particular lymphocytes and macrophages. These cells are fundamentally important because they recognise and destroy foreign macromolecules (antigens) of infectious agents and other immunogenic materials (Keast et al 1988) and thus prevent or limit infection and aid in the process of repair and recovery from injury (Newsholme et al 1988). Whilst high rates of glycolysis and glutaminolysis are characteristic features of these effector cells, it was noted that neither glucose or glutamine were fully oxidised; glucose was converted into lactate and most of the glutamine was converted to glutamate, aspartate and lactate (Ardawi and Newsholme, 1983; Newsholme et al 1987 and Ardawi, 1988). Partial oxidation of these substrates suggests that the provision of energy is not the major reason for such high rates of glycolysis and glutaminolysis. An alternative theory based on the quantitative theory of metabolic control to branched-pathways has been proposed by Newsholme et al. (1985). This theory explains how high rates of glycolysis and glutaminolysis provide metabolic intermediates for the synthesis of purine and pyrimidine nucleotides (glutamine and aspartate provide nitrogen and carbon for bases and glucose provides ribose) (Newsholme and Castell, 1996). Such biosynthesis is particularly important during an antigenic challenge. Macromolecular synthesis is required during lymphocyte proliferation and

mRNA is required by the macrophages to produce secretory proteins and phospholipid for cell membrane activity during rapid cell surface activity such as pinocytosis and phagocytosis (Newsholme et al 1988). In support of the branched point sensitivity theory, a decreased glutamine concentration in culture medium has been demonstrated to reduce lymphocyte proliferation in response to a mitogenic signal as was phagocytosis and cytokine production by macrophages (Parry-Billings et al 1990).

There is evidence which associates regular exhaustive exercise with an impairment in immune function (Newsholme and Castell, 1996), the cause of which remains elusive (Nieman, 1996 and Pedersen, 1996). As a consequence, several epidemiological studies have documented that athletes are more susceptible to URTI (Linde, 1987; Peters et al 1990, 1993 and Nieman et al 1990). It has been suggested that the *decrease in plasma glutamine* concentration as a result of prolonged physical exercise may impair the host's defence mechanisms against opportunistic infections (Newsholme et al 1985); a phenomenon which may become more frequent during an hypoxic insult. However, whilst the changes in plasma glutamine concentration *during* physical exercise are equivocal (Table 5.11), several studies have reported a decrease in plasma glutamine *following* exercise (Décombaz et al 1979; Rennie et al 1981; Babij et al 1983; Maughan and Gleeson, 1988; Parry-Billings et al 1992 and Keast et al 1995). Keast et al. (1995) demonstrated that the decrease in plasma glutamine concentrations following low and high intensity exercise was proportional to exercise intensity (r^2 = 0.97). Further evidence in support of the immunomodulatory roles of glutamine have recently been established. Chronically reduced concentrations of plasma glutamine have been reported in patients suffering from the "overtraining syndrome - OTS" (Parry-Billings et al 1990 and Rowbottom et al 1995) and chronic fatigue syndrome (Pervan et al, unpublished observations, cf Rowbottom et al, 1996), conditions which are characterised by a marked increase in the incidence of URTI and impaired wound healing.

Table 5.11 Plasma Glutamine Concentration During Submaximal (< 100%$\dot{V}O_{2max}$) and Supramaximal Exercise (> 100%$\dot{V}O_{2max}$)

Submaximal exercise		*Supramaximal exercise*	
Author (Year)	**Intensity (Change)**	**Author (Year)**	**Intensity (Change)**
Bergstrom et al ('74)	70% $\dot{V}O_{2max}$ (↑)	Katz et al ('86)	100% $\dot{V}O_{2max}$ (↑)
Babij et al ('83)	50-100% $\dot{V}O_{2max}$ (↑)	Parry-Billings ('92)	6 x 10 s sprints (↑)
Eriksson et al ('85)	80% $\dot{V}O_{2max}$ (↑)	Sewell et al ('94)	Run at 5.56 $m.s^{-1}$ (↑)
Maughan et al ('88)	70% $\dot{V}O_{2max}$ (↑)		
Sahlin et al ('90)	75% $\dot{V}O_{2max}$ (↑)		
Décombaz et al ('79)	60% $\dot{V}O_{2max}$ (↓)		
Rennie et al ('81)	50% $\dot{V}O_{2max}$ (↓)		
Parry-Billings ('92)	70% $\dot{V}O_{2max}$ (↓)		

↑ - Increase in plasma glutamine
↓ - Decrease in plasma glutamine

Sea-level concentrations of plasma glutamine obtained from subjects in the present study (PRE EXP + PRE CON - 724 ± 65 μM) were slightly higher than values obtained from other elite populations (~ 510 μM - 641 μM) reported by investigators using identical analytical techniques (Parry-Billings et al 1992). The cause of this discrepancy is unclear. The significant decrease ($P < 0.001$) in resting plasma glutamine concentration which was observed by day 19 at 1,640 m in the present study (Figure 5.8) is a novel finding which, to this author's knowledge, has not been previously reported in the literature. It was noted that two Commonwealth medallists who consistently complained of fatigue and extreme tiredness at altitude experienced the greatest decrease in plasma glutamine concentration at altitude in comparison to the remaining members of the EXP group (-250μM vs -112 ± 40μM).

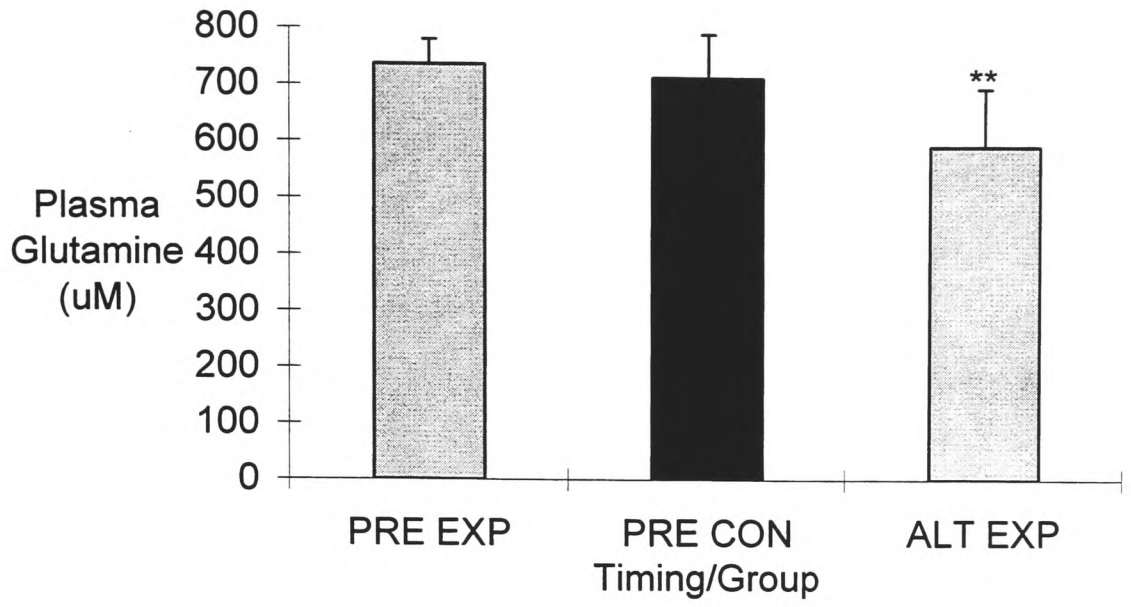


Figure 5.8 Plasma Glutamine Concentration at Sea-Level and Altitude (n = 20)
Values are Mean ± SD
**: Significantly different from within group PRE value ($P < 0.01$)
No significant difference between PRE EXP and PRE CON group means ($P > 0.05$)
PRE: Pre-altitude
ALT: Day 19 at 1,640 m
EXP: Altitude group (n = 9)
CON: Sea-level group (n = 11)

The significant decrease in EXP group mean plasma glutamine at altitude (-143 ± 74μM vs PRE, $P < 0.001$) is probably of *physiological* significance and may have contributed to the increased frequency of upper respiratory tract (URTI) and/or gastrointestinal tract infections (GTI) encountered (Figure 5.9). Communal living may have contributed to the higher incidence of infectious illness at altitude. However, it was interesting to note that there were no reports of any infectious illnesses during the study for subjects in the CON group despite identical living and training conditions.

One male 800/1,500 m specialist who was excluded from the investigation due to illness contracted an infectious illness during the altitude sojourn which continued to persist even 17 months following return to sea-level. His condition, which is at present undiagnosed, is so severe that he has been advised by clinicians to discontinue from any form of physical exercise.

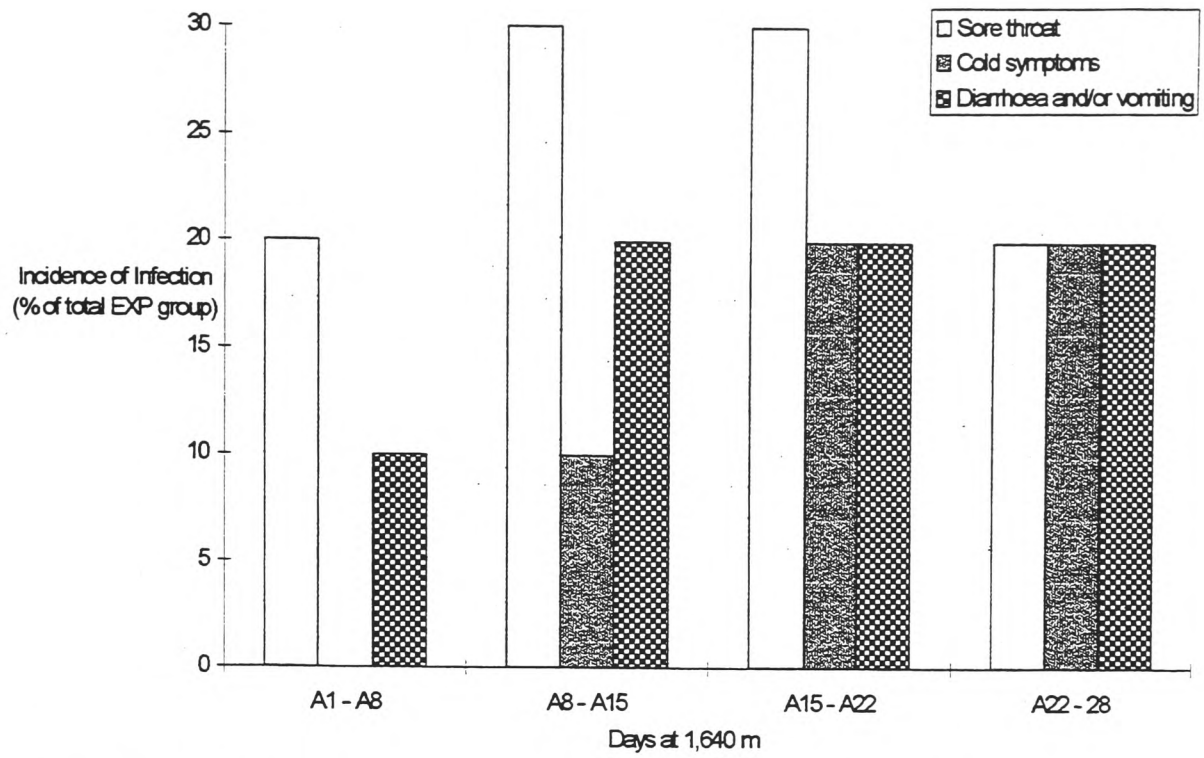


Figure 5.9 Incidence of Upper Respiratory/Gastrointestinal Tract Infections at Altitude (n = 10)
Values are Means

Based on the above findings, a model describing the relationship between ambient PO_2, training load and subsequent implications for glutamine metabolism and risk of contracting an infectious illness is presented in Figure 5.10. This model which is a modification of an original model proposed by Rowbottom et al. (1996), suggests that once a threshold hypoxic stimulus and training load is exceeded (depicted as X, termed the "metabolic crossover point") concentrations of glutamine would decrease below a physiological level thus increasing the host's susceptibility to a viral infection (termed the "window of opportunistic infection"). The critical PO_2 and training load required to exceed the metabolic crossover point await investigation.

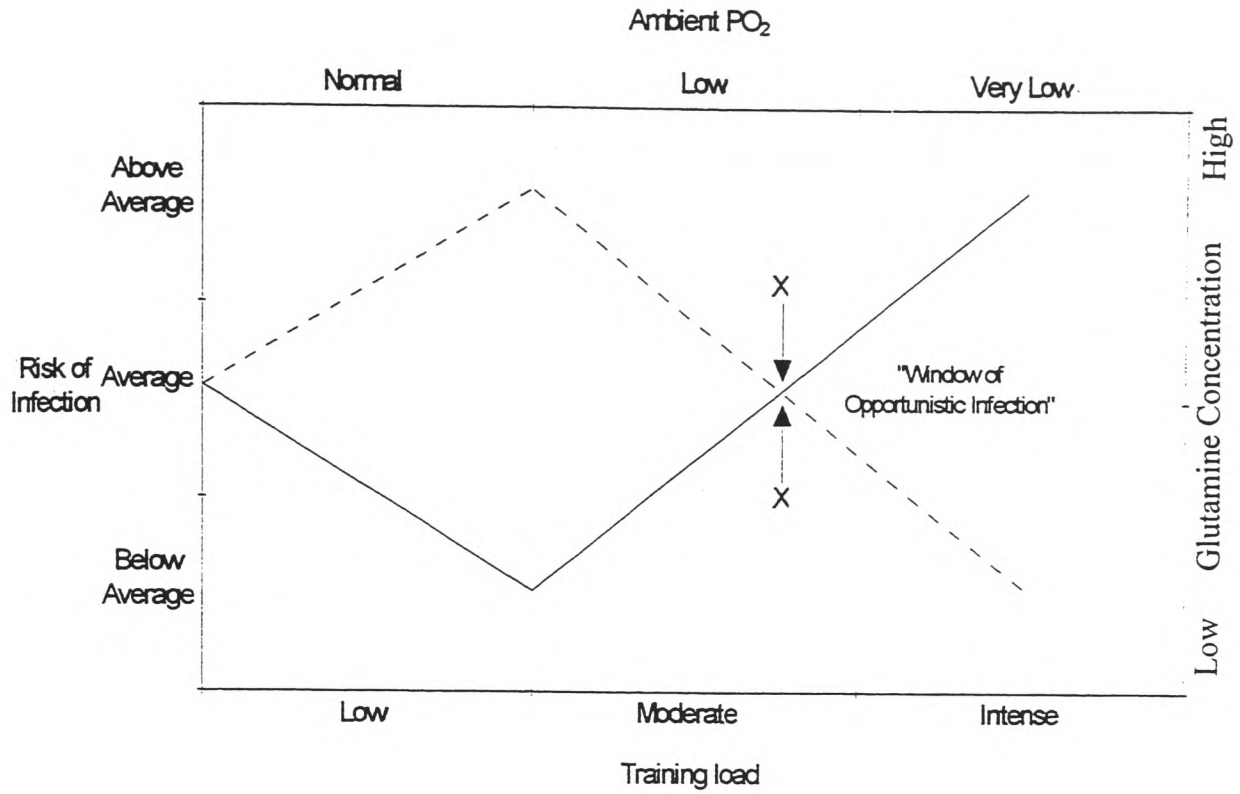


Figure 5.10 Theoretical Model Describing the Relationship Between Ambient PO_2, Training Load and Subsequent Implications for Glutamine Metabolism and Risk of Developing an Infectious Illness.

- - - - - - - - - - - Glutamine Concentration
———————— Risk of Contracting an Infectious Illness
X: Threshold stimulus or "metabolic crossover point"

Several physiological mechanisms may have been implicated in the decrease in plasma glutamine at altitude causing an imbalance between the rate of glutamine release and/or the rate of uptake by the various organs and tissues of the body. These mechanisms are discussed below:

[1] Glutamine is required by the kidney to maintain acid-base balance (Damian and Pitts, 1970; Pitts et al 1972 and Goldstein et al 1980). An increased metabolic acidosis at altitude, in particular during the early stages of acclimatisation, would therefore increase the rate of glutamine uptake.

[2] Hypoxia may have increased the hepatic uptake of glutamine. One of the liver's major roles involves the production and release of the antioxidant glutathione (Kaplowitz et al, 1985) which may require glutamine as a precursor (Snoke et al 1953 and Hong et al 1992). Hypoxia per se has been associated with an increased production of free radicals (Nagawa

et al 1968; Simon-Schnass, 1990 and Biselli et al 1992) and thus the production of free radical scavengers, such as glutathione would be expected to increase.

[3] Acute and chronic exposure to hypobaric hypoxia has been demonstrated to increase sympathoadrenergic activity with an attendant rise in circulating catecholamine concentrations (Young, 1990 and Neureither et al 1996). Wagenmakers (1990) hypothesised that glycogen depletion would result in a reduction in the availability of Krebs Cycle intemediates, in particular 2-oxoglutarate which is required for activation of the branched chain amino acid (BCAA) aminotransferase reaction which ultimately produces glutamine. However, if one considers that the normal resting concentration of glutamine in the intracellular water of human skeletal muscle is 20 $mM.L^{-1}$ and the corresponding plasma concentration is approximately 0.6 mM (Bergstrom et al 1974), the level of glycogen depletion required to decrease plasma glutamine concentrations would have to be extremely severe (personal communication, Professor E.A. Newsholme, Oxford University Biochemistry Department).

[4] Increased circulating catecholamine concentrations have been shown to decrease the rate of glutamine transport out of rat muscle incubated *in vitro* (Garber et al 1976c and Parry-Billings, 1989, unpublished data, *DPhil thesis*). Chronic hyperadrenalaemia due to shock, injury or during chronic exposure to hypoxia may have an inhibitory effect on amino acid release from skeletal muscle and thus provoke immunosupression.

The loss of skeletal muscle mass at altitude observed in the present study would suggest that glutamine production within skeletal muscle was enhanced. An increased net rate of protein degradation in skeletal muscle would provide a constant supply of branched chain amino acids which via a series of transamination reactions, would donate their nitrogen for the subsequent synthesis of glutamine (Newsholme et al 1988). This metabolic pathway may be more active during chronic exposure to hypoxia to provide glutamine for the enhanced activity of lymphocytes and macrophages. *Perhaps the catabolic effects reported in previous investigations are a useful adaptation that serve to maintain immune reactivity?*

Based on the above discussion, it is tempting to suggest that future research should incorporate BCAA and/or glutamine supplementation during their hypoxic training studies

in an attempt to maintain "normal' immune function (Bailey et al 1997) and maximise physical performance. This would effectively shift the metabolic crossover point (depicted as X) in Figure 5.9 to the right. One such study has demonstrated that oral glutamine supplementation (5 g of L-glutamine) significantly decreases an athlete's susceptibility to developing a respiratory infection at sea-level (Castell et al 1996). A recent study has also shown that in contrast to a high carbohydrate diet, BCAA supplementation attenuated skeletal muscle mass loss and peak power output during a 6 day sojourn to 2500 m - 4,100 m (Bigard et al 1996). However, whilst plasma glutamine concentrations were also shown to increase significantly following return to sea-level ($P < 0.05$, vs pre-altitude) the subsequent implications for immune function were not discussed.

An increase in the total leucocyte count and granulocyte/agranulocyte subsets provided further evidence of immunomodulation at altitude (Table 5.12). EXP group mean leucocyte count was significantly greater by day 19 to 20 at altitude ($P < 0.05$ vs PRE) due to a significant increase in neutrophil count ($P < 0.05$ vs PRE). In comparison to pre-altitude group mean values, granulocyte subsets were significantly lower in the EXP group 20 days following return to sea-level. Similar changes were also observed in the CON group subjects, the cause of which is not clear but may be related to the psychological stress invoked by impending competition. Klokker et al. (1993) has also demonstrated an increase in leucocyte count following only 20 minutes of exposure to 5,488 m, which, in contrast to the present study, they attributed to an increased concentration of *lymphocytes*. A significant decrease in lymphocyte concentration was observed following two hours of exposure to normoxic conditions ($P < 0.01$ vs hypoxic conditions). Hypoxia also induced changes in the absolute concentrations and percent distributions of blood mononuclear cell (BMNC) subpopulations; CD16+ natural killer cell activity increased whereas the percentage of CD4+ (T helper) and CD8+ (T suppressor) cells decreased. Whilst these changes did not alter the proliferative abilities of BMNC, the authors concluded that hypoxia could potentially intensify the physical symptoms associated with viral infections and cancer.

Table 5.12 Leucocyte and Differential Count at Sea-Level and Altitude (n = 20)

Cell Type	PRE EXP	ALT* EXP	$POST_2$ EXP	PRE CON	$POST_2$ CON
Leucocyte	4.8 ± 1.4	6.2 ± 1.7†	3.7 ± 1.1	5.2 ± 1.2	3.8 ± 0.9†
($x10^9$/L)	*(4.0 - 11.0)*				
Neutrophil	2.8 ± 0.9	3.7 ± 1.1†	1.1 ± 0.3‡	3.0 ± 1.2	1.1 ± 0.8‡
($x10^9$/L)	*(2.0 - 7.5)*				
Eosinophil	0.1 ± 0.2	0.2 ± 0.3	0.7 ± 0.7†	0.1 ± 0.1	0.8 ± 0.8†
($x10^9$/L)	*(0.0 - 0.7)*				
Basophil	0.0 ± 0.0	0.0 ± 0.1	0.4 ± 0.1‡	0.1 ± 0.1	0.3 ± 0.2‡
($x10^9$/L)	*(0.0 - 0.7)*				
Lymphocyte	1.4 ± 0.4	1.7 ± 0.4	1.5 ± 0.4	1.5 ± 0.4	1.6 ± 0.7
($x10^9$/L)	*(1.5 - 4.0)*				
Monocyte	0.4 ± 0.2	0.6 ± 0.5	0.4 ± 0.1	0.5 ± 0.2	0.3 ± 0.1‡
($x10^9$/L)	*(0.3 - 1.5)*				

Values are Mean ± SD
Values in italics represent reference range values
†: Significantly different from within group PRE value ($P < 0.05$)
‡: Significantly different from within group PRE value ($P < 0.01$)
PRE: Pre-altitude
ALT*: Determined during days 19 to 20 at 1,640 m
$POST_2$: Determined 20 days following EXP group return to sea-level
EXP: Altitude group (n = 8)
CON: Sea-level group (n = 12)

5.3.3.3.4 Serum Urea Concentration

Despite the hypothesised increase in the oxidation rate of BCAA at altitude in the present study, resting concentrations of serum urea did not change at altitude (Table 5.13). This would suggest, albeit rather tentatively that ammonia production was not altered by a reduction in P_IO_2. However, a definitive conclusion cannot be made without quantifying nitrogen loss by other clearance mechanisms such as urine, sweat and expired air (Graham and MacLean, 1992). In contrast, Young et al. (1987, 1992) have demonstrated that chronic exposure to altitudes ranging between 4,300 m to 7,615 m decreased plasma ammonia concentrations following both submaximal and maximal cycling exercise. However, both investigations lacked a normoxically-trained control group and thus it is quite possible that the observed shifts in substrate utilisation at altitude were independent of

hypoxia and were induced by physical training. Despite this experimental deficiency, the authors speculated that hypoxia decreased the activity of the purine nucleotide cycle (PNC), which they considered was a useful adaptation because it increased the mobilisation and oxidation of albumin bound free fatty acids (FFA), thus invoking a "glycogen sparing" effect. It is possible that the increased NH_4+ production due to an enhanced oxidation rate of the BCAA observed in the *more physically active* subjects in the present study balanced out the decrease in NH_4+ production due to an attenuated PNC activity.

Table 5.13 Resting Serum Urea Concentration at Sea-Level and Altitude (n = 20)

Variable	PRE EXP	ALT* EXP	$POST_1$ EXP	$POST_2$ EXP	PRE CON	$POST_2$ CON
n	7	7	7	7	13	13
Urea $mmol.L^{-1}$	4.59±1.45	5.48±0.51	5.67±1.38	4.97±0.72	5.65±1.37	6.12±1.17
Range	*3.38-6.19*	*4.92-6.23*	*3.73-6.89*	*3.86-5.82*	*3.35-8.34*	*4.32-8.41*

Values are Mean ± SD and *Range*
PRE: Pre-altitude
ALT*: Mean value obtained during days 15 to 27 at 1,640 m
$POST_1$: Determined 10 days following EXP group return to sea-level
$POST_2$: Determined 20 days following EXP group return to sea-level
EXP: Altitude group
CON: Sea-level group

There was an apparent dissociation between serum urea and plasma glutamine as biochemical markers of "overtraining" at altitude. Whilst plasma glutamine concentrations were significantly depressed by day 19 to 20 at altitude, resting serum urea concentrations remained stable (Figure 5.11.) with the exception of the value obtained on the 27th day which was significantly greater than the PRE EXP value ($P < 0.05$). In contrast to study 1, all subjects had resting serum urea concentrations below the threshold value of 8.5 $mmol.L^{-1}$ at altitude which, as discussed in the previous chapter has been associated with overtraining (Hollmann, 1994). Thus, it is possible that plasma glutamine is a more sensitive marker of overtraining than serum urea. The fact that several subjects contracted infectious illnesses during the altitude sojourn (Figure 5.9) would support this contention.

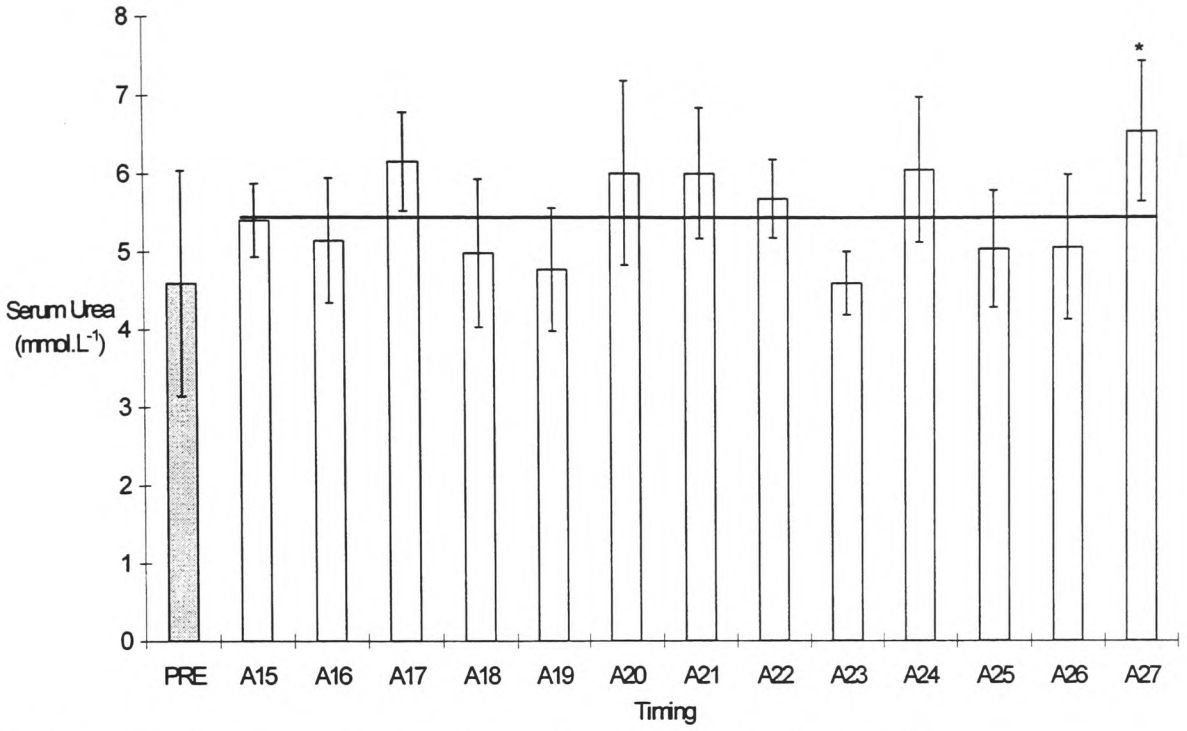


Figure 5.11 EXP Group Resting Serum Urea Concentration (n = 7)
Values are Mean ± SD
Significantly different from PRE value ($P < 0.05$)
Pre: Pre-altitude
A15 - A27: Days 15 to 27 at altitude (1640 m)
Emboldened line represents group mean serum value at altitude

5.3.3.3.5 Blood Lipid and Lipoprotein Metabolism

The physiological significance of metabolic adaptation to hypoxia is complex and poorly understood. Connett et al. (1990) have recently reviewed the major biochemical "subsystems", namely oxidative phosphorylation, the Krebs cycle, glycolysis, substrate supply and cell energetics and how they interact via signals from pH and P_i to match ATP demand and aerobic ATP production. This homeostatic mechanism serves to prevent true skeletal muscle O_2-limited cytochrome turnover termed "dysoxia" and thus defend intracellular PO_2. These compensatory mechanisms have been shown to defend ATP homeostasis in the working vastus lateralis muscle even during acute and chronic exposure to 4,300 m (Green et al 1992).

Potentially "useful" adaptations to hypoxia include an increased glucose uptake by working skeletal muscle possibly due to an increase in hexokinase activity (Cooper et al 1986; Katz and Sahlin, 1989 and Green et al 1992) and an enhanced mobilisation and oxidation of FFA during chronic exposure (Young et al 1982, 1987 and Braun et al 1997). Whilst the evidence is equivocal, data from epidemiological and cross-sectional studies suggest that

the enhanced lipolytic effects of environmental hypoxia may invoke cardioprotective changes in blood lipid-lipoprotein metabolism (Section 2.5.4.5.). The physiological mechanisms of action are poorly understood and wait further investigation, in particular the potentially regulating effect of hypoxia on the major lipid-regulatory enzymes which include: lipoprotein lipase (LPL), lecithin:cholesterol acyltransferase (LCAT), hepatic triglyceride lipase (HTGL), in addition to cholesterol ester transfer protein (CETP). Endurance training has been demonstrated to increase the mobilisation and oxidation rate of FFA, thus reducing the re-esterification rate of FFA into triglyceride (Klein et al 1994). Could hypoxia potentiate this process and thus decrease the hepatic release of triglyceride rich lipoproteins? A summary of the potential biochemical adaptations that may enhance blood lipid-lipoprotein metabolism is depicted in Figure 5.12.

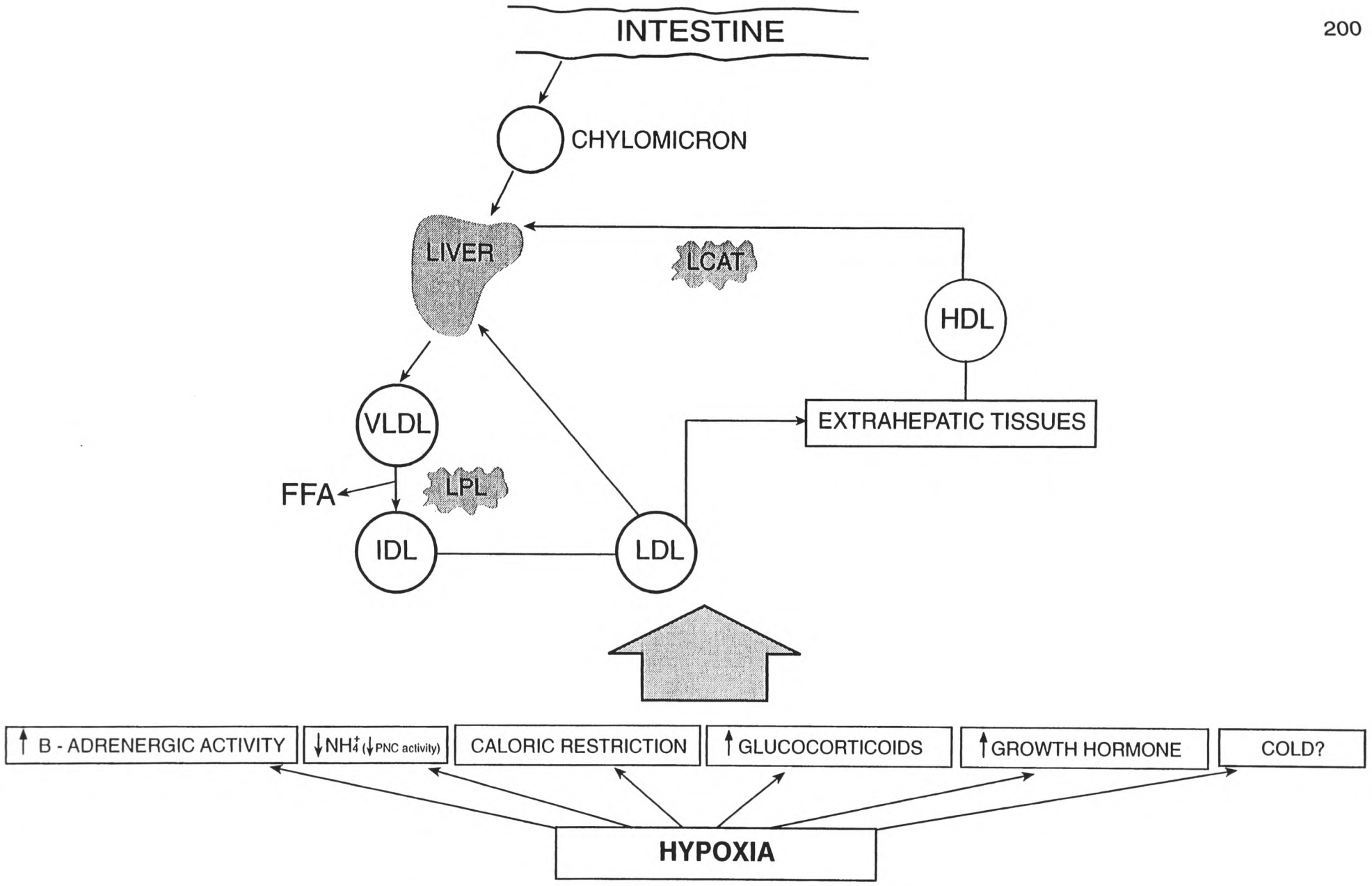


Figure 5.12 Lipid-Lipoprotein Metabolism and Hypoxia

However, field based studies have failed to incorporate a normoxically trained control group in the experimental design and thus it is difficult to isolate the metabolic effects of hypoxia from those induced by increases in physical activity. Whilst a control group was incorporated in the present study, the results presented in Table 5.14 are by no means definitive. Nutrient intake was not controlled *throughout* the entire experimental period and it is possible that total energy expenditure differed between groups, in particular whilst the EXP group trained at altitude. However, personal experience would suggest that control of these potentially confounding variables is extremely difficult in particular when International athletes are incorporated in the experimental design. With these experimental limitations in mind, the data do not indicate any evidence of hypoxia-mediated changes in blood lipid-lipoprotein metabolism.

Table 5.14 Fasting Serum Lipid and Lipoprotein Concentrations at Sea-Level (n = 16)

Variable	PRE EXP	$POST_2$ EXP	PRE CON	$POST_2$ CON
Total Cholesterol ($mmol.L^{-1}$)	4.03 ± 0.60 *(< 5.20)*	3.97 ± 0.66	4.48 ± 0.52	4.09 ± 0.59
Triglyceride ($mmol.L^{-1}$)	0.94 ± 0.15 *(0.50 - 1.75)*	1.06 ± 0.34	0.80 ± 0.28	0.78 ± 0.13
Total/HDL-C ($mmol.L^{-1}$)	3.37 ± 0.40 *(< 6.00)*	3.41 ± 0.27	3.19 ± 0.60	3.00 ± 0.74
HDL-C ($mmol.L^{-1}$)	1.20 ± 0.11 *(> 0.90)*	1.17 ± 0.21	1.43 ± 0.15	1.41 ± 0.24
LDL-C ($mmol.L^{-1}$)	2.41 ± 0.54 *(< 4.0)*	2.32 ± 0.42	2.69 ± 0.56	2.32 ± 0.59
Apolipoprotein A ($mg.dl^{-1}$)	133 ± 10 *(♂ 94 - 175)*	135 ± 14 (♀ 101 - 199)	149 ± 11	149 ± 10
Apolipoprotein B ($mg.dl^{-1}$)	76.6 ± 14.4 *(♂ 52 - 109)*	79.5 ± 8.7 (♀ 49 - 103)	77.1 ± 16.5	69.6 ± 17.2
Lipoprotein (a) ($mg.dl^{-1}$)	42.2 ± 56.5 *(Not defined)*	54.0 ± 76.5	23.7 ± 28.6	23.1 ± 28.5

Values are Mean ± SD
Bracketed values in italics indicate "suggested" healthy values
PRE: Pre-altitude
$POST_2$: Determined 20 days following EXP group return to sea-level
EXP: Altitude group (n = 6)
CON: Sea-level group (n = 10)

The initially normal blood lipid-lipoprotein levels of subjects involved in the present study may have precluded any hypoxia-mediated changes in fat metabolism. A recent meta-analysis of 27 studies has identified that higher initial concentrations of total cholesterol (5.2 $mmol.L^{-1}$ to 6.2 $mmol.L^{-1}$) induce more effective lipid changes (Lokey and Tran, 1989). A greater reduction in cholesterol and triglyceride concentration can also be expected if training is accompanied by weight loss (Lokey and Tran, 1989). As illustrated in Figure 5.5, Table 5.6, weight loss, in particular of body fat was *not* observed in the present study.

It is also conceivable that a more severe hypoxic stimulus was required (lower ambient PO_2/increased duration) to potentiate the metabolic modulation of lipid metabolism.

Several investigations have reported a dose-response relationship between weekly running distance and HDL-C, triglyceride and cholesterol concentration (Lakka and Salonen, 1992 and Kokkinos et al 1995). This has profound implications for the risk of coronary heart disease mortality which has been demonstrated to decrease by 3.5% per 0.03 $mmol.L^{-1}$ increase in HDL-C (Gordon et al 1989). However, no significant correlations between either pooled pre-altitude EXP and CON group mean weekly running distance or $\dot{V}O_{2max}$ and metabolic indices of lipid-lipoprotein metabolism were observed in the present study. Resting indices of blood lipid-lipoprotein concentration were comparable to values reported by other studies that have employed slightly less active and aerobically fit distance runners (Table 5.15).

Table 5.15 Resting Blood Lipid-Lipoprotein Concentrations in Elite Distance Runners

Dependent Variable	Stein et al (1991)	Hortobagyi et al (1993)	*Present Study*
n	105	12	*18*
Age (Years)	22 ± 4	20 ± 1	*24 ± 4*
Body Mass (kg)	69.0 ± 6.6	65.5 ± 8.3	*66.3 ± 7.5*
Running Distance ($km.week^{-1}$)	No data	73 ± 17	*89 ± 23*
$\dot{V}O_{2max}$ ($ml.kg.^{-1}\ min.^{-1}$)	68.6 ± 6.8	61.0 ± 7.0	*68.8 ± 10.7*
Total Cholesterol ($mmol.L^{-1}$)	4.28 ± 0.58	4.24 ± 0.56	*4.03 ± 0.60*
Triglyceride ($mmol.L^{-1}$)	1.07 ± 0.35	0.79 ± 0.25	*0.94 ± 0.15*
HDL-C ($mmol.L^{-1}$)	1.18 ± 0.04	1.23 ± 0.21	*1.20 ± 0.11*
LDL-C ($mmol.L^{-1}$)	2.80 ± 0.57	2.48 ± 0.39	*2.41 ± 0.54*

Values are Mean ± SD

5.3.3.4 Cardiovascular Adaptations

Group mean systolic and diastolic blood pressure and mean arterial blood pressure (MABP) did not change during the study (Table 5.16).

Table 5.16 Resting Blood Pressure at Sea-Level and Altitude (n = 20)

	Systolic/Diastolic Pressure (mmHg)		MABP (mmHg)	
Timing	**EXP**	**CON**	**EXP**	**CON**
PRE	124 ± 7 / 71 ± 5	116 ± 11/ 72 ± 10	88 ± 4	87 ± 9
ALT*	120 ± 7 / 47 ± 26	.....	79 ± 7	.....
$POST_1$	120 ± 13 / 59 ± 13	.....	79 ± 8	.....
$POST_2$	115 ± 11 / 69 ± 8	118 ± 9/ 74 ± 10	84 ± 7	88 ± 7

Values are Mean ± SD
PRE: Pre-altitude
ALT*: Mean value obtained during days 18 to 27 at 1,640 m
$POST_1$: Determined 10 days following EXP group return to sea-level
$POST_2$: Determined 20 days following EXP group return to sea-level
EXP: Altitude group (n = 7)
CON: Sea-level group (n = 13)

Unlike the previous study, no changes were observed in resting blood pressure on consecutive days at 1,640 m. The cause of this discrepancy is unclear.

Resting HR has previously been demonstrated to increase during both acute and chronic altitude exposure due to increased β-adrenergic drive and a decreased stroke volume (SV) due to reduced cardiac filling (Reeves et al 1987). In contrast, the present study demonstrated that EXP group mean supine HR *decreased* significantly by 5 $b.min^{-1}$ ($P < 0.01$ vs PRE) during the altitude sojourn and remained depressed following 10 days return to sea-level (Table 5.17). The cause of this decrease is interesting. It is unlikely to reflect an increased SV; subjects were already at the peak of physiological adaptation and as discussed previously, hypoxia is more likely to have induced a *decrease* in SV. Hypoxia may have altered the resting parasympathetic/sympathetic tone, with the parasympathetic

system predominating at rest. Whilst only a speculation, this condition may have indicated that the subjects were suffering from a "parasympathetic form" of overtraining. Parasympathetic overtraining has been identified in elite distance runners following an experimentally-induced increase in weekly running distance from 86 ± 15 km to 175 ± 27 km during a 3 week period (Lehmann et al 1991). This represents an advanced stage of overtraining (Stone et al 1991) and might indicate a decreased intrinsic sympathetic activity due to hypothalamic dysfunction (Barron et al 1985) and/or a reduced sensitivity to catecholamines which has been observed in chronic hyperstimulation or exhaustive stress (Brodde et al 1984 and Tohmeh and Cryer, 1980). Whilst further research is required to qualify this hypothesis, the additional stress of hypoxia may have accelerated this process.

Table 5.17 Heart Rate (HR) at Sea-Level and Altitude (n = 20)

	Supine HR ($b.min^{-1}$)		Standing HR ($b.min^{-1}$)		Δ HR ($b.min^{-1}$)	
Timing	**EXP**	**CON**	**EXP**	**CON**	**EXP**	**CON**
PRE	53 ± 6	50 ± 5	63 ± 4	64 ± 9	10 ± 9	14 ± 8
ALT*	48 ± 4‡	.....	81 ± 8‡	.....	33 ± 9‡	.....
$POST_1$	49 ± 6†	.....	64 ± 8	.....	16 ± 5	.....
$POST_2$	56 ± 6	53 ± 8	77 ± 9†	67 ± 8	21 ± 11	14 ± 7

Values are Mean ± SD
†: Significantly different from within group PRE value ($P < 0.05$)
‡: Significantly different from within group PRE value ($P < 0.01$)
PRE: Pre-altitude
ALT*: Mean value obtained during days 15 to 27 at 1,640 m
$POST_1$: Determined 10 days following EXP group return to sea-level
$POST_2$: Determined 20 days following EXP group return to sea-level
EXP: Altitude group (n = 7)
CON: Sea-level group (n = 13)

Despite the predominance of parasympathetic activity in the supine position, the sympathetic response to orthostatic stress was enhanced at altitude (Table 5.17, Figure 5.13). An improvement in orthostatic tolerance (↑HR/↑MABP) has previously been reported during 8 days at 3,255 m due to an increase in sympathetic tone (Loepkky et al

1993). However, it is also possible that an increase in free radical mediated nitric oxide (NO) release may have been implicated in the greater increase in group mean standing HR at altitude (+18 $b.min^{-1}$ vs PRE, $P < 0.01$) observed in the present study. Enhanced peripheral vasodilation would have resulted in a greater pressure decrease at the baroreceptor site (carotid artery) due to an increased pooling of blood in the lower extremities.

As a consequence, group mean ΔHR increased by 23 $b.min^{-1}$ at altitude ($P < 0.01$ vs PRE) which as previously discussed in Study 1 would suggest that subjects were in an overtrained state. Thus, it would appear that either hypoxia and/or physiological changes associated with overtraining are responsible for complex changes in the autonomic and hormonal regulation of the sympathetic/parasympathetic nervous systems. The significance of these changes is poorly understood but may represent a control mechanism which ultimately regulates the delivery of oxygen to skeletal muscle.

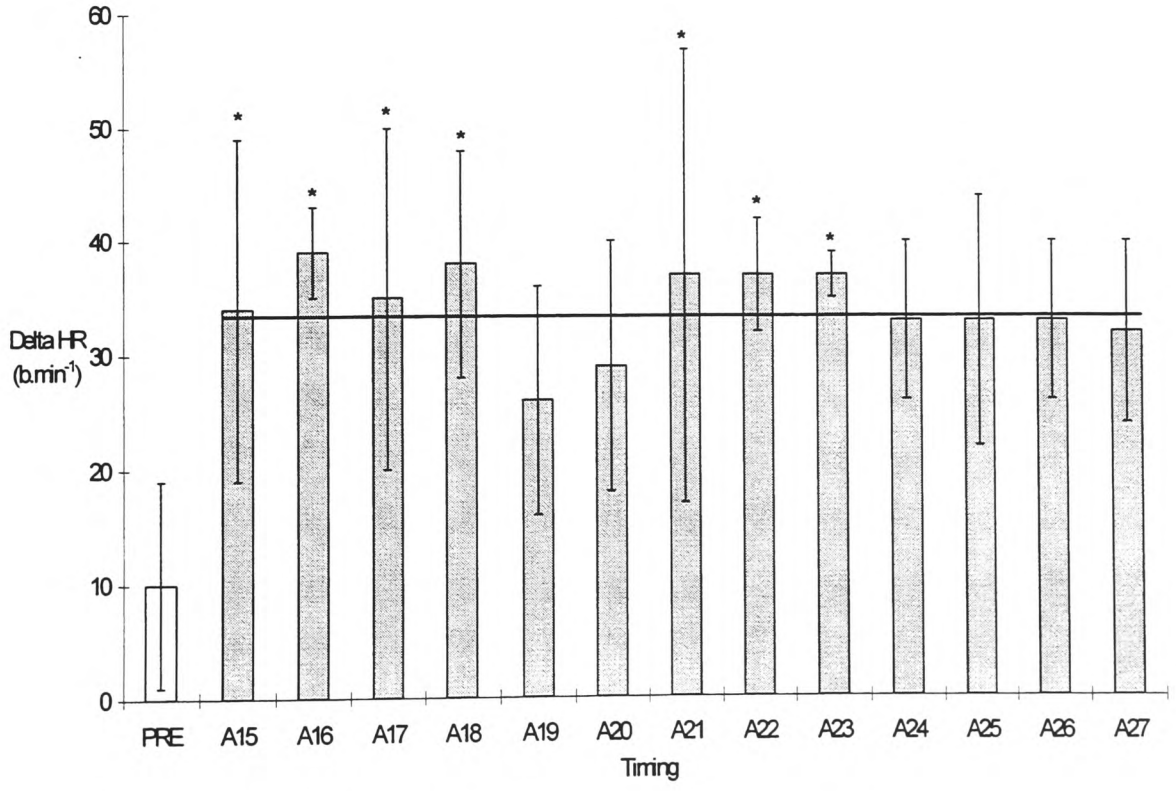


Figure 5.13 Modified Orthostatic Stress Test at Altitude (n = 7)
Values are Mean ± SD
PRE: Pre-altitude
A15 - A27: Days 15 to 27 at 1,640 m
*: Significantly different from PRE value ($P < 0.05$)
Emboldened line represents group mean value at altitude

5.3.3.5 Respiratory Adaptations

Lung function data obtained at sea-level and on day 16 at 1,640 m are summarised in Table 5.18. Lung volumes appeared healthy and comparable to predicted values for sedentary subjects of the same age, sex, height and weight. However, mid-expiratory flow rates ($FEF_{25-75\%}$) were significantly lower than predicted values (EXP - 87 ± 22% of predicted value, $P < 0.05$ / CON - 84 ± 19% of predicted value, $P < 0.05$), an observation which has previously been noted in elite subjects (personal communication, Dr M.Harries, consultant respiratory physician, Northwick Park Hospital, UK).

A reduction in air density at altitude has been implicated in altered respiratory mechanics, in particular a decrease in viscous airways resistance (Hollmann et al 1994). A recent investigation by Forte et al. (1997) demonstrated significant increases in MVV and FEV_1 at 4,300 m and as a consequence, maximum sustainable ventilation (MSV) increased by 15% ($P < 0.001$ vs normoxia). This is likely to influence endurance performance as it has been suggested that the MSV (horizontal $\dot{V}_E$ asymptote) represents the highest pulmonary ventilation that can be maintained without a sustained and progressive metabolic acidosis of the respiratory muscles (Whipp and Ward, 1994). However, it was apparent that the 19% decrease in air density at 1,640 m (Shephard, 1992) did not alter lung function and was therefore unlikely to have influenced exercise performance.

Table 5.18 Lung Function Data

Variable	PRE EXP	ALT* EXP	$POST_2$ EXP	PRE CON	$POST_2$ CON
n	7	7	7	13	13
FVC - L	5.28 ± 0.95	5.49 ± 0.86	5.31 ± 0.92	5.15 ± 0.71	5.18 ± 0.71
FEV_1 - L	4.43 ± 0.91	4.55 ± 0.88	4.38 ± 0.85	4.21 ± 0.62	4.21 ± 0.62
FEV_1/FVC (%)	84 ± 6	83 ± 7	83 ± 6	75 ± 22	82 ± 6
PEF - $L.min^{-1}$	9.78 ± 1.86	10.36 ± 1.86	9.77 ± 1.85	9.10 ± 2.42	9.60 ± 1.93
$FEF_{25-75\%}$ $L.min^{-1}$	4.59 ± 1.36	4.62 ± 1.41	4.40 ± 1.41	4.16 ± 1.12	4.09 ± 1.10
MVV $L.min^{-1}$	179 ± 38	193 ± 39	176 ± 39	156 ± 39	165 ± 39

Values are Mean ± SD.

FVC: Forced vital capacity
FEV_1: Forced expiratory volume (1s)
PEF: Peak expiratory flow rate
$FEF_{25-75\%}$: Mid-expiratory flow rate
MVV: Maximum Voluntary Ventilation
PRE: Pre-altitude
ALT*: Altitude (Measurements conducted on day 16 at 1,640 m)
$POST_2$: Determined 20 days following EXP group return to sea-level
EXP: Altitude group
CON: Sea-level group

5.3.4 Laboratory Measurements

The following sections will present and discuss the metabolic and cardiorespiratory responses to laboratory and track exercise at sea-level and altitude (1,640 m). Three subjects in the EXP group were excluded from the overall analyses due to lower limb stress fractures which were diagnosed by the 3rd week at altitude.

5.3.4.1 Environmental Conditions

Ambient temperature and relative humidity remained stable during the study (Table 5.19). However, barometric pressure decreased by approximately 126 mmHg at 1,640 m which represented a 13% decrease in P_IO_2 (151 mmHg to 125 mmHg).

Table 5.19 Environmental Conditions During Laboratory Testing

Variable	PRE	ALT*	$POST_1$	$POST_2$
Temperature (°C)	24 ± 0	27 ± 2	24 ± 1	22 ± 0
	(24 - 24)	*(25 - 28)*	*(23 - 25)*	*(22 - 22)*
Relative Humidity (%)	50 ± 0	41 ± 3	48 ± 1	33 ± 1
	(49 - 50)	*(38 - 45)*	*(47 - 50)*	*(32 - 33)*
Barometric Pressure (mmHg)	768 ± 4	642 ± 1	753 ± 0	768 ± 3
	(752 - 771)	*(641 - 643)*	*(753 - 753)*	*(758 - 770)*

Values are Mean ± SD and *Range*
PRE: Pre-altitude
ALT*: Determined between days 19 to 20 at 1,640 m
$POST_1$: Determined 10 days following EXP group return to sea-level
$POST_2$: Determined 20 days following EXP group return to sea-level

5.3.4.2 Metabolic Adaptations

There were no significant differences observed in whole blood lactate concentration ($[La^-]_B$) on immediate completion of and 3 minutes following recovery from a $\dot{V}O_{2max}$ test as a function of timing (Table 5.20). Table 5.21 also demonstrates that the increase in $[La^-]_B$ following a 3 minute walk recovery at 1.39 ms^{-1} ($\Delta[La^-]_B$) also remained unchanged during the study. These data contrast with the findings of other studies which have demonstrated significant reductions (relative to pre-altitude and acute altitude values) in submaximal and maximal lactate concentrations following acclimatisation to 4,300 m to 5,050 m (Young et al 1982; Bender et al 1989; Young et al 1992; Kayser et al 1994 and Grassi et al 1995). A decrease in substrate flux through oxidative phosphorylation mediated by a central limitation of power output (Kayser et al 1994) and changes in the adrenergic sensitivity of glycolysis (Brooks et al 1992) appear to be implicated in what has been termed the "lactate paradox" (Section 2.5.4.3.). It is theoretically possible that the decrease in P_IO_2 (-13% vs PRE) encountered in the present study was insufficient for activation of the above mentioned central and peripheral mechanisms.

Table 5.20 Whole Blood Lactate Concentration ($[La^-]_B$) During and 3 Minutes Following Recovery From a $\dot{V}O_{2max}$ Test

Timing	Peak $[La^-]_B$ ($mmol.L^{-1}$)		Recovery $[La^-]_B$ ($mmol.L^{-1}$)	
	EXP (n = 7)	CON (n = 12)	EXP (n = 7)	CON (n = 12)
PRE	6.08 ± 1.84	6.29 ± 0.95	8.18 ± 1.76	8.24 ± 1.73
ALT*	5.55 ± 0.70	.....	7.63 ± 1.46	.....
$POST_1$	6.79 ± 0.39	.....	8.80 ± 1.30	.....
$POST_2$	7.24 ± 2.76	6.99 ± 1.33	8.64 ± 2.06	8.84 ± 1.43

Values are Mean ± SD

PRE: Pre-altitude

ALT*: Determined between days 19 to 20 at 1,640 m

$POST_1$: Determined 10 days following EXP group return to sea-level

$POST_2$: Determined 20 days following EXP group return to sea-level

EXP: Altitude group

CON: Sea-level group

Table 5.21 Increase in Whole Blood Lactate Concentration [($\Delta[La^-]_B$): Recovery Value - Peak Value] Following Completion of a $\dot{V}O_{2max}$ Test (n = 19)

Timing	EXP (Δ $[La^-]_B$ ($mmol.L^{-1}$)	CON (Δ $[La^-]_B$ ($mmol.L^{-1}$)
PRE	2.10 ± 0.50	1.95 ± 1.62
ALT*	2.08 ± 0.81	.....
$POST_1$	2.01 ± 1.23	.....
$POST_2$	1.55 ± 1.07	1.85 ± 0.76

Values are Mean ± SD
PRE: Pre-altitude
ALT*: Determined between days 19 to 20 at 1,640 m
$POST_1$: Determined 10 days following EXP group return to sea-level
$POST_2$: Determined 20 days following EXP group return to sea-level
EXP: Altitude group (n = 7)
CON: Sea-level group (n = 12)

5.3.4.3 Cardiovascular Adaptations

Using a one legged training model, Terrados et al. (1990) demonstrated significantly lower submaximal HR's during a normobaric cycling test following 4 weeks of hypobaric training as opposed to an equivalent training program performed in normobaria. Whilst this response was not discussed by the authors, it is possible that an enhanced peripheral oxygen extraction due to an increased citrate synthase activity and myoglobin content (measured) and/or an increased stroke volume (not measured) may have been implicated in the observed bradycardia. However, submaximal HR (and RPE) did not change as a function of timing during the present study (Table 5.22). As previously demonstrated in study 1, both EXP and CON groups consistently underperceived exercise intensity, in this case by approximately 44 $b.min^{-1}$ (Figure 5.14).

Table 5.22 Heart Rate (HR) and Ratings of Perceived Exertion (RPE) During Individual Stages of an Incremental Treadmill Test to Exhaustion

Group	EXP (n = 7)		CON (n = 12)	
Timing	**Stage 1**	**Stage 2**	**Stage 1**	**Stage 2**
PRE HR ($b.min^{-1}$)	167 ± 10	184 ± 13	168 ± 11	179 ± 9^
ALT* HR ($b.min^{-1}$)	164 ± 9	173 ± 5	.....	.....
$POST_1$ HR ($b.min^{-1}$)	170 ± 8	184 ± 8	.....	.....
$POST_2$ HR ($b.min^{-1}$)	165 ± 10	180 ± 9	167 ± 10	178 ± 9^
PRE RPE	11 ± 1	14 ± 1	12 ± 1	14 ± 2^
ALT* RPE	12 ± 1	15 ± 2	.....	.....
$POST_1$ RPE	13 ± 1	16 ± 2	.....	.....
$POST_2$ RPE	12 ± 0	15 ± 1	11 ± 3	15 ± 2^

Values are Mean ± SD
^: Significantly different from preceding mean value ($P < 0.05$)
PRE: Pre-altitude
ALT*: Determined between days 19 to 20 at 1,640 m
$POST_1$: Determined 10 days following EXP group return to sea-level
$POST_2$: Determined 20 days following EXP group return to sea-level
EXP: Altitude group
CON: Sea-level group

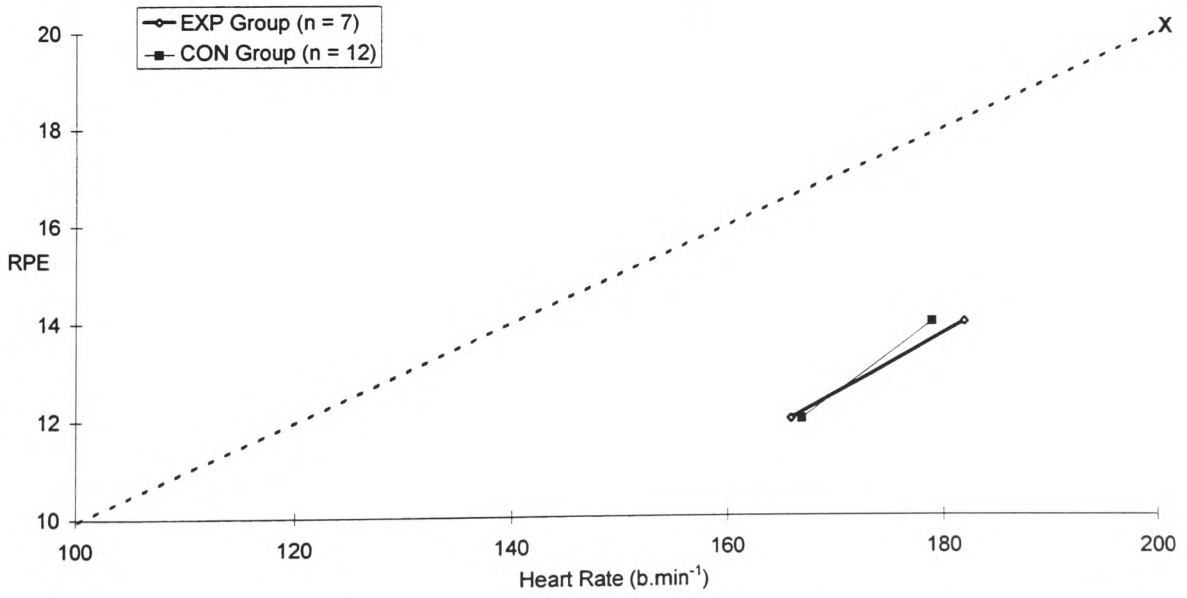


Figure 5.14 Relationship Between Submaximal Heart Rate (HR) and Ratings of Perceived Exertion (RPE)
Values are Mean
Each data point represents pooled PRE and POST group means during each treadmill stage
Line of identity (denoted as X) represents the "normal" relationship between HR and RPE

A summary of the heart rate response immediately after and 3 minutes following a $\dot{V}O_{2max}$ test are presented in Table 5.23. In comparison to pre-altitude values, maximal or recovery HR did not change during sea-level tests. The *rate* of HR recovery (ΔHR: maximal-recovery value) also remained unchanged during the study. However, maximal HR was significantly decreased ($P < 0.01$) during days 19 to 20 at altitude (Table 5.23).

Table 5.23 Maximal and Recovery Heart Rate Following a $\dot{V}O_{2max}$ Test at Sea-Level and Altitude (n = 19)

Variable	PRE	ALT*	$POST_1$	$POST_2$
EXP HR_{MAX} ($b.min^{-1}$)	187 ± 8	175 ± 6‡	191 ± 7	190 ± 6
EXP HR_{REC} ($b.min^{-1}$)	130 ± 17	108 ± 8	128 ± 12	123 ± 11
EXP ΔHR (Maximal-Recovery)	57 ± 16	68 ± 3	63 ± 17	67 ± 6
CON HR_{MAX} ($b.min^{-1}$)	189 ± 12	.....	.....	184 ± 11
CON HR_{REC} ($b.min^{-1}$)	119 ± 16	.....	.....	116 ± 13
CON ΔHR (Maximal-Recovery)	70 ± 20	.....	.....	68 ± 10

Values are Mean ± SD
‡: Significantly different from within group PRE value ($P < 0.01$)
HR_{MAX}: Maximum heart rate
HR_{REC}: Recovery heart rate
PRE: Pre-altitude
ALT*: Determined between days 19 to 20 at 1,640 m
$POST_1$: Determined 10 days following EXP group return to sea-level
$POST_2$: Determined 20 days following EXP group return to sea-level
EXP: Altitude group (n = 7)
CON: Sea-level group (n = 12)

A decrease in HR_{MAX} has been observed in previous investigations, the magnitude of which depends on the duration and intensity of the hypoxic stimulus (Saltin, 1988 and Savard et al 1995). A decrease in HR_{MAX} of 10 $b.min^{-1}$ has been observed in elite athletes training intensively at moderate altitudes equivalent to ~2,000 m (Saltin et al 1996 and Svedenhag, unpublished observations). An increase in vagal activity and/or a down-regulation of β-receptor function may be implicated in the decreased chronotropic drive of the heart during prolonged exposure to altitude (Richalet et al 1988 and Savard et al 1995). However, the significance of this response is poorly understood, despite its first description by Christensen and Forbes in 1937.

Ward et al. (1995) have suggested that the decrease in maximal HR at altitude may be the consequence of a decreased maximal work output. A decrease in maximal work output was observed in the present study (Table 5.24), a direct result of a pulmonary diffusion limitation due to a decreased alveolar PO_2 as evidenced by an SaO_2 of 82 ± 5% (Range: 74% - 85%) which was observed at the point of physical exhaustion. However, the decrease in maximal HR may represent a more "useful" adaptation which serves to optimise $\dot{V}_A/\dot{Q}_C$ at altitude (Saltin, 1996). Any increase in maximal HR would decrease pulmonary capillary transit time (PCTT) and thus *further* decrease PaO_2 at altitude (Section 2.5.4.3).

Table 5.24 Running Time To Exhaustion During a $\dot{V}O_{2max}$ Test (n = 19)

Group (n)	PRE	ALT*	$POST_1$	$POST_2$
EXP (n = 7)	485 ± 23	383 ± 37†	460 ± 19	475 ± 31
CON (n = 12)	539 ± 105	.....	.....	501 ± 99

Values are Mean ± SD
†: Significantly different from within group PRE value ($P < 0.05$)
PRE: Pre-altitude
ALT*: Determined between days 19 to 20 at 1,640 m
$POST_1$: Determined 10 days following EXP group return to sea-level
$POST_2$: Determined 20 days following EXP group return to sea-level
EXP: Altitude group
CON: Sea-level group

Throughout this investigation, we observed that HR_{MAX} was repeatedly shown to be only 139 $b.min^{-1}$ at *sea-level* in a 27 year old male 5000 m specialist (Commonwealth Games gold medallist). This value was approximately 42 to 66 $b.min^{-1}$ lower than his age predicted value assuming a HR_{MAX} of 193 ± 12 $b.min^{-1}$. It was therefore clear that other central or peripheral adaptations had to exist to account for his superior physical performance capabilities despite marked chronotropic insufficiency. In an attempt to elucidate potential mechanisms, an echo-cardiac ultrasound was performed. A summary of the results is presented in Appendix L.

Whilst there was evidence of mild uniform left ventricular hypertrophy (Left ventricle posterior wall in diastole = 13 mm), it was surprising to note that intra-cardiac dimensions and blood flow kinetics at rest were within the upper limits of normal and consistent with

other observations in elite athletes. It is unlikely that the maximum arterial-venous oxygen difference (A-aO_2 diff) was markedly greater than values attained by other subjects involved in the present study. Application of the Fick equation would therefore suggest that the predominant adaptation would be increased SV_{MAX}, possibly due to enhanced myocardial contractility and/or increased left ventricular end-diastolic volume mediated by an enhanced cardiac filling time. This is demonstrated mathematically below:

1. Assuming a *normal* HR_{max} [220 - Age (Years)] for a 27 year old male of *193 b.min^{-1}* and an A-aO_2 $diff_{max}$ of *0.16 l.min^{-1}* (Rowell, 1986).

Transposition of Fick's equation::

$$SV_{max} = \frac{\dot{V}O_{2max}}{HR_{max} \times A\text{-}vO_2\ diff_{max}}$$

$$SV max = \frac{4.83\ (L.min^{-1})}{193\ (b.min^{-1}) \times 0.16\ (L.min^{-1})} = \mathbf{156\ ml.bt^{-1}}$$

2. Assuming a *low* HR_{max} of 139 *b.min^{-1}*:

$$SV max = \frac{4.83\ (L.min^{-1})}{139\ (b.min^{-1}) \times 0.16\ (L.min^{-1})} = \mathbf{217\ ml.bt^{-1}}$$

Much interest has focused on whether an increased SV_{MAX} allows for a decreased HR_{MAX} or vice versa (Wilmore and Costill, 1994). Depressed HR_{MAX}'s have been previously observed in elite athletes and it has been suggested that the increase in SV and lower HR is the most efficient means of achieving a given cardiac output (Wilmore and Costill, 1994). The decreased HR_{MAX} may *also* represent a regulatory mechanism which serves to optimise $\dot{V}_A/\dot{Q}_C$ during maximal exercise even at sea-level. An alveolar-capillary diffusion limitation has been demonstrated during maximal exercise at sea-level in elite athletes (Koistinen et al 1995) and thus any further increase in $\dot{Q}$ would effectively increase arterial hypoxaemia. Whatever the significance of this response, the low maximum HR observed in the present study has not been previously documented in the literature and warrants further investigation (personal communication, Professor B.Whipp, St George's Hospital Medical School, UK).

5.3.4.4 Respiratory Adaptations

A summary of the respiratory responses during and following recovery from an incremental treadmill test to exhaustion is presented in Table 5.25 and Table 5.26. EXP group mean $\dot{V}O_{2max}$ values expressed in both absolute and relative terms was 13% lower following 19 to 20 days at 1,640 m ($P < 0.05$ vs PRE). The magnitude of this decrease was identical to an *extrapolated value* based on Terrados et al's (1992) data using *elite* athletes

$$y = -0.0079\,x + 100.07\ (r^2 = 0.99)$$

where:

y - percentage of $\dot{V}O_{2max}$ determined in normoxia
x - altitude in metres

Previous investigators have demonstrated that the decrement in $\dot{V}O_{2max}$ determined in hypoxia is linearly related to $\dot{V}O_{2max}$ determined in normoxia (Lawler et al 1988; Martin and Kroy, 1993 and Koistinen et al 1995) such that the more "aerobically fit" athletes experience the greatest gas exchange impairments. However, no significant correlation was observed between $\Delta\dot{V}O_{2max}$ (ALT EXP - PRE EXP) and PRE EXP $\dot{V}O_{2max}$ in the present study ($r = -0.31$, $P = 0.68$).

The decrease in $\dot{V}O_{2max}$ and SaO_2 (82 ± 5%) at altitude occurred despite a 31.2 L.min^{-1} increase in $\dot{V}_E$ ($P < 0.01$ vs PRE). A classical hypobaric chamber study by Sutton et al. (1988) also demonstrated that despite a 52 L.min^{-1} increase in $\dot{V}_E$ (BTPS) during a 60W increment in cycling power output on the "summit" of Mt Everest, CaO_2 decreased by 7 ml.L^{-1} due to a 1.3 mmHg increase in the [A-a] O_2 diff. The role of ventilation-perfusion ($\dot{V}_A/\dot{Q}_C$) inequality and alveolar-end-capillary diffusion limitation in the widening of the [A-a] O_2 diff during exercise at altitude (3049 m to 8840 m) has been investigated by Wagner et al. (1986, 1987). Using the multiple inert gas elimination technique (Wagner et al 1974), the authors demonstrated that $\dot{V}_A/\dot{Q}_C$ inequality increased during exercise at altitude possibly due to an accumulation of interstitial perivascular and peribronchial fluid mediated by an increase in pulmonary arterial pressure. Préfaut et al. (1997) have recently speculated that the increased capillary transmural pressure experienced by elite athletes during exercise would induce stress failure with endothelial breaks. This process would initiate the release of inflammatory mediators, in particular histamine, thus causing interstitial fluid accumulation and arterial hypoxaemia. This response would further increase the alveolar-end-capillary diffusion limitation due to the decreased PAO_2 (Wagner et al

1986, 1987) and thus account for the observed hypoxaemia experienced by subjects in the present study.

Table 5.25 Respiratory Responses During Maximal Treadmill Exercise at Sea-Level and Altitude

Group	EXP (n = 7)				CON (n = 12)	
Variable	**PRE**	**ALT***	**$POST_1$**	**$POST_2$**	**PRE**	**$POST_2$**
$\dot{V}O_2$ ($L.min^{-1}$)	5.15 ± 0.95	4.48±0.53†	5.21 ± 0.67	5.10 ± 0.59	4.43 ± 0.95	4.43 ± 0.8
$\dot{V}O_2$ ($ml.kg^{-1}min^{-1}$)	74.5 ± 6.4	63.8 ± 2.6†	76.0 ± 2.7	73.7 ± 3.4	69.0 ± 10.2	69.0 ± 8.0
$\dot{V}_E/\dot{V}O_2$ ($L.min^{-1}$)	28.9 ± 1.7	39.8 ± 2.2‡	26.1 ± 1.6	28.6 ± 1.7	27.4 ± 3.9	28.0 ± 3.5
$\dot{V}_E$ ($L.min^{-1}$)	147.9±20.4	179.1±30.1‡	136.8±25.8	145.3±14.5	119.5±22.1	122.9±22.5
RER	1.13 ± 0.06	1.11 ± 0.04	1.11 ± 0.01	1.17 ± 0.03	1.13 ± 0.08	1.15 ± 0.05

Values are Mean ± SD
Gas volumes expressed at standard temperature pressure dry (STPD)
†: Significantly different from within group PRE value ($P < 0.05$)
‡: Significantly different from within group PRE value ($P < 0.01$)
PRE: Pre-altitude
ALT*: Determined between days 19 to 20 at 1,640 m
$POST_1$: Determined 10 days following EXP group return to sea-level
$POST_2$: Determined 20 days following EXP group return to sea-level
EXP: Altitude group
CON: Sea-level group

Table 5.26 Respiratory Responses During Recovery from Maximal Treadmill Exercise at Sea-Level and Altitude

Group	EXP (n = 7)				CON (n = 12)	
Variable	PRE	ALT*	$POST_1$	$POST_2$	PRE	$POST_2$
$\dot{V}O_2$ ($L.min^{-1}$)	1.71 ± 0.45	1.74 ± 0.16	1.64 ± 0.34	1.59 ± 0.34	1.41 ± 0.35	1.34 ± 0.24
$\dot{V}O_2$ ($ml.kg^{-1}min^{-1}$)	24.7 ± 5.5	24.9 ± 2.9	23.7 ± 1.7	22.8 ± 2.1	21.9 ± 4.2	20.9 ± 2.3
$\dot{V}_E/\dot{V}O_2$ ($L.min^{-1}$)	35.3 ± 3.6	51.8 ± 3.3‡	38.9 ± 4.3	42.6 ± 4.0	36.1 ± 8.4	38.2 ± 8.6
$\dot{V}_E$ ($L.min^{-1}$)	59.6 ± 14.2	90.4 ± 14.0‡	64.9 ± 20.8	67.8 ± 16.4	49.6 ± 13.3	49.9 ± 7.8
RER	1.21 ± 0.13	1.24 ± 0.06	1.29 ± 0.10	1.31 ± 0.05	1.25 ± 0.16	1.31 ± 0.11

Values are Mean ± SD
Gas volumes expressed at standard temperature pressure dry (STPD)
‡: Significantly different from within group PRE value ($P < 0.01$)
PRE: Pre-altitude
ALT*: Determined between days 19 to 20 at 1,640 m
$POST_1$: Determined 10 days following EXP group return to sea-level
$POST_2$: Determined 20 days following EXP group return to sea-level
EXP: Altitude group
CON: Sea-level group

5.3.5 Track Measurements

A summary of the environmental conditions experienced during a standardised track session conducted at sea-level and on day 18 at 1,640 m is presented in Table 5.27. Ambient temperature, relative humidity and wind velocity remained reasonably stable throughout the study which would suggest that changes in the physiological responses to supramaximal exercise were most likely mediated by hypoxia *per se*.

Table 5.27 Environmental Conditions During Track Testing

Variable	PRE	ALT*	$POST_2$
Temperature (°C)	17.5 - 22.0	18.5 - 19.5	13.0 - 19.6
Relative Humidity (%)	47 - 50	38 - 40	31 - 36
Barometric Pressure (mmHg)	769 - 771	629 - 630	769 - 770
Wind Velocity ($m.sec^{-1}$)	1.7 - 1.9	1.9 - 2.4	1.8 - 2.1

Values are Ranges
PRE: Pre-altitude
ALT*: Determined on day 18 at 1,640 m
$POST_2$: Determined 20 days following EXP group return to sea-level

The physiological responses to a standardised track session at sea-level and altitude are summarised in Tables 5.28 to 5.30. Five subjects in the EXP group were excluded from the overall analyses due to injury. In contrast, CON group subjects remained injury-free and compliance was maximal.

5.3.5.1 Metabolic Adaptations

A summary of the changes in group mean $[La^-]_B$ as a function of repetition number and timing is presented in Table 5.28. In general, an increased lactacidosis was observed between repetition 1 and 2 for both groups, indicating that the rate of lactate production predominantly by skeletal muscle exceeded the rate of clearance via oxidation and/or gluconeogenesis (Roth, 1991). Whilst CON group mean $[La^-]_B$ concentrations continued to increase by the 3rd and final repetition, EXP group mean $[La^-]_B$ attained a *peak* value by the end of the 2nd repetition, the cause of which is unclear. However, peak $[La^-]_B$ concentrations were not significantly different between groups, suggesting that the maximal rate of ATP derivation from glycolysis or glycogenolysis was comparable.

Table 5.28 Changes in Whole Blood Lactate Concentration ($[La^-]_B$) During a Standardised Track Session at Sea-Level and Altitude (n = 18)

Variable	Group/Timing	Repetition 1	Repetition 2	Repetition 3	Repetition X
Rep $[La^-]_B$	PRE EXP	5.69 ± 0.42	8.90 ± 0.36^	9.21 ± 0.29	*7.93 ± 0.23*
$mmol.L^{-1}$	ALT* EXP	4.53 ± 0.29	8.07 ± 0.45^	8.97 ± 0.67	*7.19 ± 0.39*
	$POST_2$ EXP	4.26 ± 1.32	8.96 ± 1.19^	9.97 ± 1.46	*7.73 ± 1.26*
	PRE CON	5.16 ± 1.26	7.23 ± 1.31^	8.68 ± 1.67^	*7.02 ± 1.13*
	$POST_2$ CON	5.46 ± 1.55	8.48 ± 1.26^†	9.79 ± 1.76^†	*7.91 ± 1.37*

Values are Mean ± SD

^: Significantly different from preceding mean value ($P < 0.05$)

†: Significantly different from within group PRE value ($P < 0.05$)

PRE: Pre-altitude

ALT*: Determined on day 18 at 1,640 m

$POST_2$: Determined 20 days following EXP group return to sea-level

EXP: Altitude group (n) = 5

CON: Sea-level group (n) = 13

However, relative to pre-altitude group mean values, there were no significant differences observed in EXP group mean repetition $[La^-]_B$ at altitude and 20 days following return to sea-level. In contrast, CON group $[La^-]_B$ was significantly greater during the 2nd and 3rd repetitions ($P < 0.05$ vs PRE) which signified either an increased efflux of $[La^-]_B$ across the sarcolemma into the blood and/or a decreased rate of clearance. However, mean $\Delta[La^-]_B$ ($POST_2$ - PRE values) did not differ between groups (EXP: -0.20 $mmol.L^{-1}$ ± 1.45 $mmol.L^{-1}$ vs CON: 0.88 $mmol.L^{-1}$ ± 1.71 $mmol.L^{-1}$). The increase in $[La^-]_B$ was probably of minor physiological significance as it did not potentiate the fatigue process as evidenced by an unchanged running velocity (Table 5.30).

A significant decrease in total weekly running distance in preparation for a major competition (Figure 5.4 and Table 5.4) may have caused an alteration in lactate kinetics. However, it was intriguing that the EXP group mean $[La^-]_B$ did not change despite a similar decrease in total weekly running distance. Thus, hypoxia may have invoked central/peripheral adaptations that either increased the rate of lactate clearance or decreased the rate of lactate efflux from skeletal muscle by increasing oxygen flux from the capillaries to the mitochondria. Evidence for an increased rate of lactate clearance has been demonstrated at altitude due to an increased rate of lactate consumption by skeletal

muscle, liver and in particular the heart (Raynaud et al 1974 and Fellmann et al 1988). The mechanism (s) responsible for this response may have persisted following return to sea-level. The rate of lactate production by skeletal muscle may also have decreased due to a shift in the enzyme isoform of lactate dehydrogenase (LDH) from LDH_{4-5} (muscle isoform) to LDH_{1-2} (heart isoform) which would favor the oxidation of lactate to pyruvate (Saltin, 1996). Activation of the malate-aspartate shuttle system which transports NADH electrons from the cytosol into the mitochondria (Schantz et al 1986) may also have increased. Perhaps activation of these mechanisms occurred only during and following *supramaximal* exercise as substrate flux did not change during laboratory-based *maximal* exercise (Section 5.3.4.2.)

5.3.5.2 Cardiovascular Adaptations

Table 5.29 summarises the changes in repetition and group mean HR, recovery HR (30 seconds post-repetition) and RPE during the track session. In contrast to the depressed EXP group mean HR_{max} values obtained during a $\dot{V}O_{2max}$ test at altitude (Table 5.23), peak repetition HR (~HR_{max}) remained unchanged, a paradoxical finding which is difficult to explain. One may speculate that lower peak repetition HR's may have attained statistical significance at altitude if the sample size was larger. The 50% dropout rate observed in the present study significantly reduced the power of statistical testing and thus a greater change would have been required before statistical significance was observed. There were no changes observed in EXP and CON group mean recovery HR's as a function of timing. Whilst EXP group mean RPE did not change at altitude or following return to sea-level, CON group mean RPE was significantly greater following termination of the 2nd repetition ($P < 0.01$ vs PRE) probably due to an increased lactacidosis (Table 5.28).

Table 5.29 Heart rate (HR) and Ratings of Perceived Exertion (RPE) During a Standardised Track Session (n = 18)

Variable	Group/Timing	Repetition 1	Repetition 2	Repetition 3	Repetition X
Repetition	PRE EXP	177 ± 7	180 ± 12	187 ± 10	*181 ± 10*
HR	ALT* EXP	172 ± 7	179 ± 5^	184 ± 6	*178 ± 6*
($b.min^{-1}$)	$POST_2$ EXP	179 ± 5	187 ± 9	189 ± 9	*185 ± 8*
	PRE CON	176 ± 10	182 ± 11^	184 ± 11	*181 ± 10*
	$POST_2$ CON	178 ± 10	182 ± 11^	184 ± 11^	*181 ± 10*
Recovery	PRE EXP	138 ± 6	137 ± 16	.....	*138 ± 10*
HR	ALT* EXP	129 ± 22	138 ± 18	.....	*134 ± 20*
($b.min^{-1}$)	$POST_2$ EXP	138 ± 5	137 ± 15	.....	*138 ± 9*
	PRE CON	125 ± 10	128 ± 14	.....	*127 ± 11*
	$POST_2$ CON	127 ± 12	131 ± 12^	.....	*129 ± 11*
RPE	PRE EXP	13 ± 1	14 ± 1	15 ± 2	*14 ± 1*
	ALT* EXP	15 ± 1	17 ± 1	18 ± 1	*17 ± 0*
	$POST_2$ EXP	14 ± 2	16 ± 2^	17 ± 2	*16 ± 2*
	PRE CON	13 ± 1	14 ± 1	16 ± 2^	*14 ± 1*
	$POST_2$ CON	13 ± 2	15 ± 2^‡	17 ± 2^	*15 ± 2†*

Values are Mean ± SD

^: Significantly different from preceding mean value ($P < 0.05$)

†: Significantly different from within group PRE value ($P < 0.05$)

‡: Significantly different from within group PRE value ($P < 0.01$)

PRE: Pre-altitude

ALT*: Determined on day 18 at 1,640 m

$POST_2$: Determined 20 days following EXP group return to sea-level

EXP: Altitude group (n) = 5

CON: Sea-level group (n) = 13

5.3.5.3 Running Performance

EXP group mean running velocity decreased at altitude ($P < 0.01$ vs PRE) most likely caused by a diffusion limitation due to the lower PAO_2 (Table 5.30). It was interesting to note that running velocity was also decreased following return to sea-level ($P < 0.05$ vs PRE) which would suggest that the additional stress of hypobaric hypoxia had a *negative effect* on supramaximal exercise performance. In contrast to CON group data, the lack of an increase in EXP group mean $[La^-]_B$ and RPE during $POST_2$ testing may suggest that the subjects were not performing to the best of their abilities. However, the near maximum HR values obtained during each repetition would strongly suggest that this was not the case. The decreased running velocity also appeared to be independent of any changes in environmental conditions, in particular wind velocity (Table 5.19). Whilst not quantified, a decrease in the absolute training intensity performed at altitude is the most likely cause of what appears to constitute a "detraining response". Levine et al. (1997) have recently demonstrated the importance of maintaining absolute training intensity at altitude if performance potentiating effects are to be elicited following return to sea-level. However, in light of the findings of the present thesis, the physiological and perhaps ethical implications of maintaining such high workloads at altitude by elite athletes need to be re-considered, in particular when one considers the increased potential for physical injury and adverse changes in immune function.

Table 5.30 Running Velocity During a Standardised Track Session (n = 18)

Variable	Group/Timing	Repetition 1	Repetition 2	Repetition 3	Repetition X
Rep Velocity ($m.s^{-1}$)	PRE EXP	5.84 ± 0.07	5.80 ± 0.11	5.74 ± 0.13	*5.79 ± 0.10*
	ALT* EXP	5.68 ± 0.13	5.50 ± 0.13†	5.46 ± 0.17	*5.55 ± 0.13‡*
	$POST_2$ EXP	5.69 ± 0.21	5.67 ± 0.10	5.65 ± 0.10	*5.67 ± 0.09†*
	PRE CON	5.62 ± 0.51	5.61 ± 0.56	5.65 ± 0.58	*5.63 ± 0.55*
	$POST_2$ CON	5.67 ± 0.48	5.62 ± 0.51	5.61 ± 0.52	*5.63 ± 0.50*

Values are Mean ± SD
†: Significantly different from within group PRE value ($P < 0.05$)
‡: Significantly different from within group PRE value ($P < 0.01$)
PRE: Pre-altitude
ALT*: Determined on day 18 at 1,640 m
$POST_2$: Determined 20 days following EXP group return to sea-level
EXP: Altitude group (n) = 5
CON: Sea-level group (n) = 13

5.4 SUMMARY

A common feature of both the present and previous study was the decrease in sample size due to either subject injury and/or illness. The compliance rate in the present study decreased by 50% and thus conclusions should be considered tentatively owing to the statistical limitations of non-parametric statistical analyses.

The physiological responses observed in the present study were essentially independent of changes in environmental conditions and training load, and most likely associated with the additional stress of hypobaric hypoxia. Previous investigations have failed to control for these potentially confounding variables and thus the validity of their experimental conclusions are questionable.

Four weeks of controlled physical training at 1,640 m did not confer any additional physiological advantage to that achieved by a comparable program of training conducted at sea-level. It was also observed that physiological indices of maximal and supramaximal running performance were not affected by the timing of sea-level testing. In contrast, supramaximal exercise was *impaired* following return to sea-level conditions as noted by a significant decrease in group mean running velocity during the performance of a standardised track session. A decrease in the absolute training intensity performed at altitude due to a decreased ambient PO_2 was the most likely cause of this response.

The physiological mechanisms responsible for the general lack and potentially adverse effects of altitude training on sea-level performance were to some extent elucidated in the present study. Four subjects in the altitude training group were diagnosed as iron deficient at sea-level (serum ferritin stores ~ < 35 ng/ml) which may have been implicated in the lack of haematological adaptation during the altitude sojourn. A significant decrease in resting plasma glutamine concentration and an increased incidence of upper respiratory tract and gastrointestinal infections were also observed at altitude. This would suggest, albeit tentatively, that hypoxia per se was associated with adverse changes in immune function. A novel model has been proposed which describes the importance of maintaining plasma glutamine concentrations above a "physiological level" to maintain normal immune reactivity at altitude when the competitor is challenged with the combined stresses of an intensive training load and a decreased P_IO_2. Oral supplementation with branched chain

amino acids (BCAA) or glutamine could serve to maintain normal immune function and thus attenuate the incidence of subclinical infection at altitude.

CHAPTER 6

SYNTHESIS OF FINDINGS

6. SYNTHESIS OF FINDINGS

This chapter will summarise and discuss the major findings of the studies outlined in this thesis. Initially, a critical review of the experimental limitations encountered in Study 1 and Study 2 will be discussed. Subsequent implications for the realisation of aims will be considered prior to an examination of the original null hypotheses. This will be followed by a general discussion which will consider the physiological implications of altitude training for the health and fitness of the elite competitor. Finally, a series of practical guidelines that are designed to optimise physiological acclimatisation to environmental hypoxia will be presented prior to concluding remarks.

6.1 EXPERIMENTAL LIMITATIONS

A 30% drop-out rate was anticipated in the present research and incorporated into the calculation of the power of statistical testing. However, due to a high incidence of injury and/or infectious illness encountered during both altitude sojourns, the compliance rate decreased in some instances by 60%. As a consequence, *some* experimental data were not normally distributed and were thus analysed using non-parametric statistics. The limitations of non-parametric statistics are recognised (Altman, 1991) and the conclusions formulated by the present research should therefore be considered with caution. Based on these experimental findings, it is suggested that investigators should allow for a 60% drop-out rate if future research into hypoxia is to be conducted successfully using elite athletes.

6.2 REALISATION OF AIMS

Examination of the null hypotheses in the present thesis required the realisation of four experimental aims that were originally formulated at the close of Chapter 2. These are outlined and discussed below:

Aim [1]: *To evaluate the effects of hypobaric hypoxia on physiological indices of exercise performance in a cohort of elite distance runners.*

Aim [2] *To determine the implications of chronic exposure to hypobaric hypoxia on physiological correlates of exercise performance following return to sea-level.*

Aim [3] *To examine factors that are potentially implicated in the modulation of exercise performance at altitude and following return to sea-level.*

International standard distance runners (800 m to 10 km specialists) were recruited in conjunction with the British Athletics Federation and the British Olympic Association. These subjects attended two sea-level and altitude training camps (New Mexico, USA and Krugersdorp, S.Africa) which were organised over a twelve month period. Quantification of the physiological adaptations invoked during 4 weeks of altitude acclimatisation were established by repeated field-based testing which was conducted in "portable" altitude-based laboratories. Laboratory and field-based exercise tests were devised to assess the effects of hypobaric hypoxia on physiological indices of *submaximal*, *maximal* and *supramaximal* running performance at altitude and following return to sea-level. The influence of subject iron status and the timing of the descent to sea-level as potential modulators of exercise performance were also examined.

Aim [4] *To investigate the potentially adverse physiological responses to hypobaric hypoxia and subsequent implications for the fitness and health of the elite competitor.*

A marked increase in the frequency of upper respiratory and gastrointestinal tract infections was observed at altitude and the subsequent diagnosis of infectious mononucleosis in two male subjects shortly following return to sea-level stimulated an investigation into the potentially adverse effects of hypobaric hypoxia on physiological function at rest and during exercise. Specific cardiovascular and metabolic markers of "exercise stress" were monitored at altitude as a means of assessing physiological adaptation to the additional stress of hypobaric hypoxia. The *in vivo* immunological response to hypobaric hypoxia was determined indirectly by measuring resting plasma glutamine concentration.

6.3 TESTING OF NULL HYPOTHESES (H_O)

The following section will consider six null hypotheses which were tested by this thesis.

Null Hypothesis [1]: *Hypobaric hypoxia does not affect the physiological response during and following recovery from maximal exercise.*

This null hypothesis was rejected. Running time to volitional exhaustion decreased by 21% between days 19 to 20 at 1,640 m ($P < 0.05$ vs pre-altitude mean). Maximal heart rate was 12 b.min^{-1} lower at altitude ($P < 0.01$ vs pre-altitude mean). Maximal oxygen consumption ($\dot{V}O_{2max}$) expressed in both absolute and relative terms was 14% lower at altitude ($P < 0.05$ vs pre-altitude). Maximum minute ventilation ($\dot{V}_E$) and the ventilatory equivalent for oxygen ($\dot{V}_E/\dot{V}O_2$) increased significantly by 31.2 L.min^{-1} (STPD) and 10.9 L.min^{-1} (STPD) respectively at altitude ($P < 0.01$ vs pre-altitude means). Mean values for $\dot{V}_E$ and $\dot{V}_E/\dot{V}O_2$ increased by 30.8 L.min^{-1} (STPD) and 16.5 L.min^{-1} (STPD) respectively following a 3 minute recovery at altitude ($P < 0.01$ vs pre-altitude means).

Null Hypothesis [2]: *Hypobaric hypoxia does not affect physiological responses during and following revovery from supramaximal exercise.*

In comparison to pre-altitude group mean values, running velocity during a standardised track session was 3% lower at 1,500 m ($P < 0.05$) and 4 % lower at 1,640 m ($P < 0.05$). This null hypothesis was therefore rejected.

Null Hypothesis [3]: *Four weeks of continuous exposure to hypobaric hypoxia does not improve physiological indices of submaximal performance 3 weeks following return to sea-level.*

A decreased lactacidosis during submaximal exercise was observed in the altitude-trained group only following return to sea-level ($P < 0.05$ vs pre-altitude mean). However, this decrease fell within the pre-determined critical difference for this metabolite and an unchanged lactate threshold (θ $[La^-]_B$) suggested that the decrease in whole blood lactate concentration was physiologically insignificant. There were no significant differences in any

other cardiovascular or respiratory markers of "running economy" following return to sea-level.

Altitude training was associated with a significant increase in the incidence of injury and/or infectious illness. As a consequence, forty percent of the altitude trained group were unable to perform exercise even 3 weeks following return to sea-level. Physiological indices of submaximal running performance were *adversely* affected in one male subject who was diagnosed with infectious mononucleosis shortly after returning from the altitude sojourn. In light of these findings, the null hypothesis was therefore accepted.

Null Hypothesis [4]: *Physiological performance during and following recovery from maximal exercise at sea-level are not improved following four weeks of continuous exposure to hypobaric hypoxia.*

This null hypothesis was accepted. Relative to pre-altitude group mean values, selected metabolic, cardiovascular and respiratory measurements obtained during and following recovery from a standardised treadmill test to volitional exhaustion did not change following *either* 10 or 20 days return to sea-level.

Null Hypothesis [5]: *Four weeks of continuous exposure to hypobaric hypoxia does not potentiate physiological indices of supramaximal exercise performance following return to sea-level.*

The null hypothesis was accepted. Relative to pre-altitude group mean values, continuous exposure to 1,640 m, (equivalent to a 29 mmHg decrease in P_IO_2) group mean running velocity was significantly *lower* following 3 weeks return to sea-level ($P < 0.05$).

Null Hypothesis [6]: *Immune function is not altered by chronic hypobaric hypoxia.*

In comparison to sea-level baseline values, there was a marked increase in the frequency of infectious illnesses (upper respiratory and gastrointestinal tract infections) contracted during the altitude sojourns. Fifty percent of the subjects in the New Mexico (n = 7) and S.Africa (n = 5) studies contracted infectious illnesses during the altitude sojourn. Two male subjects who had contracted an upper respiratory tract (URT) and gastrointestinal tract

(GT) infection during the altitude sojourn to New Mexico were subsequently diagnosed with infectious mononucleosis shortly following return to sea-level.. The physical symptoms of one male subject who contracted an infectious illness during the S.Africa sojourn continued to persist even 17 months following return to sea-level. He has been advised to discontinue any forms of physical exercise due to the severity of his condition. Resting concentrations of plasma glutamine, an indirect measure of *in vivo* immunoreactivity, decreased at altitude ($P < 0.001$ vs pre-altitude mean). Thus, in light of these findings, the null hypothesis was rejected.

6.4 GENERAL DISCUSSION: PHYSIOLOGICAL ADAPTATIONS INVOKED BY CHRONIC HYPOBARIC HYPOXIA

Two separate investigations were conducted at the same time of year during a twelve month experimental period. A two-group repeated-measures experimental design was employed to investigate the effects of 4 weeks exposure to hypobaric hypoxia on physiological indices of submaximal, maximal and supramaximal exercise performance at altitude and following return to sea-level. The physiological implications of altitude training for the *health* of the subject were also investigated, with particular reference to hypoxia-mediated immunosupression and the subsequent risks of contracting an infectious illness.

A substantial number of hypoxic-training studies have failed to *isolate* the independent effects of hypoxia per se on physiological function at rest and during exercise which compromises experimental conclusions (Bailey and Davies, 1997). Mountainous environments impose other physiological stresses such as changes in training load, diet and environmental conditions which confound those induced by hypoxia (Houston et al 1987 and Young et al 1989). Specific attention therefore focused on the incorporation of a performance-matched normoxically-trained control group in the research described in this thesis. Attempts were made to match the training load of both the altitude and sea-level trained groups. Elite athletes were employed as experimental subjects because of a professional and almost religious adherence to their training lifestyles. It is suggested that because these subjects are most likely at the peak of physiological adaptation, any changes in physiological function at altitude and following return to sea-level could be attributed to the independent effects of hypoxia.

6.4.1 Physiological Responses at Rest

Secondary polycythaemia has been strongly implicated in the performance-potentiating effects of altitude training following return to sea-level (Berglund, 1992). However, this contention is *not* absolute as the relationship between an increased arterial oxygen content (CaO_2) and the utilisation of oxygen by working skeletal muscle is complicated by reductions in blood flow mediated by increases in vascular resistance and/or regional or systemic vasoconstriction (Chapter 2). Whilst oxygen transport was not quantified in the present study due to the absence of blood flow measurements, it was clear that CaO_2 did not change appreciably due to the stable Hb concentrations. Sakai et al. (1994) have also

demonstrated unchanged resting Hb concentrations following 5 weeks of exposure to intermittent hypobaric hypoxia at an ambient PO_2 equivalent to 1,500 m. In contrast, secondary polycythaemia has been demonstrated by other investigations which have been conducted at similar altitudes over similar time periods using elite athletes (Klausen et al 1991; Ingjer and Mhyre, 1992 and Roberts and Smith, 1992). Differences in the plasma volume response to the independent stimuli of exercise and hypoxia may be a contributory factor that is implicated in these contradictory findings. It is quite possible that the observed secondary polycythaemia was the consequence of a haemoconcentration. This would certainly provide a contributory explanation as to why Klausen et al. (1991) did not observe any improvements in $\dot{V}O_{2max}$ despite an elevated reticulocytosis and increase in Hb concentration. In contrast, stable urine osmolalities and a positive fluid balance would suggest that there were no major fluid losses experienced in the present study. Whilst only a speculation, is it possible that these subjects were able to "defend" plasma volume more effectively than subjects involved in the afore-mentioned studies?

It is conceivable that the hypoxic stimulus (intensity and/or duration) experienced by the subjects in the present study was not sufficient to activate haematological adaptation. Weil et al. (1968) have demonstrated an inflection point in red blood cell mass in resting subjects based at altitude when the PaO_2 decreased to 67 mmHg, equivalent to an interpolated SaO_2 of 92% (Chapter 2). The "threshold" hypoxic stimulus required to invoke this level of arterial desaturation would equate to an ambient PO_2 encountered between 2,200 m to 2,500 m (Levine and Stray-Gundersen, 1992). Thus, it has been suggested that subjects should be based at this threshold altitude and exposure to lower altitudes would be futile as it is unlikely to invoke physiological adaptation (Levine and Stray-Gundersen, 1992). This *belief* would certainly explain why so few altitude training studies have been conducted at lower altitudes equivalent to those encountered in the present study. However, these suggestions have been formulated using sedentary subjects at rest. *If* a low PaO_2 is the critical stimulus that conveys messages to the central nervous system "signaling" for physiological adaptation, then it is likely that the elite subject who is more prone to developing arterial hypoxaemia than a lesser-trained counterpart (Préfaut et al 1997) would desaturate *during exercise* and thus benefit from training at significantly lower altitudes.

Few studies have quantified iron kinetics and subsequent haematological adaptation at altitude and following return to sea-level (Reynafarje et al 1959 and Reynafarje and Ramos,

1961). Depressed iron stores (quantified as serum ferritin concentration) such as those encountered in the present study prior to the S.Africa altitude sojourn may have suppressed haemopoiesis. Despite widespread oral iron supplementation equivalent to 200 mg per day of ferrous sulphate, fifty seven percent of the subjects in the altitude-trained group and sixty percent of the subjects in the sea-level trained group had serum ferritin concentrations below 35 ng/ml most likely indicative of prelatent iron deficiency. Logistical limitations precluded the administration of parenteral iron treatment prior to the altitude sojourn to normalise subject iron stores. It is therefore possible that the already depressed iron stores could not match the marked increase in iron demand for the synthesis of Hb at altitude.

Specific attempts were made to quantify the potentially adverse effects of chronic exposure to hypobaric hypoxia and the subsequent implications for physiological function at rest and during exercise. The potential implications for altered biomechanics due to the mountainous environment at altitude and the subsequent risk of musculo-skeletal injury is a consideration that has eluded scientific investigation. However, the incidence of injury in the present research tended to increase at altitude probably due to changes in the environmental terrain, in particular due to the lack of soft running surfaces. This posed a particular problem for a male and female subject who suffered lower limb stress fractures during the S.Africa altitude sojourn which subsequently prevented them from participating in the 1996 Olympic Games that were held in Atlanta, USA.

Hypoxia appeared to be associated with alterations in resting parasympathetic/sympathetic tone and cardiovascular data suggested that the additive stress of hypobaric hypoxia *may* have induced an "overtrained" response in subjects at altitude. Consistent underperception of exercise intensity at altitude may indicate that the *elite* athlete is more susceptible to this condition. There was also evidence for adverse changes in immune function at altitude. The frequency of physical symptoms associated with URT and GI infections tended to increase at altitude. Two male subjects who suffered URT/GI infections during the New Mexico altitude sojourn were subsequently diagnosed with infectious mononucleosis shortly following return to sea-level. The onset of physical symptoms that are characteristic of the first 3 to 5 days of the prodrome suggested that the subjects were exposed to the Epstein-Barr virus during the initial stages of altitude acclimatisation. This may suggest that the potentially immunosupressive effects of environmental hypoxia are most pronounced during the early stages of physiological adaptation presumably when the host is most vulnerable to

attack from viral pathogens. Detailed medical and physiological assessments demonstrated that one of these subjects recovered fully from the condition within 10 months of diagnosis. In contrast, the physical symptoms of the other subject were so severe that he did not return to competitive racing until 12 months following the initial diagnosis. The mechanisms responsible for the increased frequency of infectious illness at altitude were to some extent elucidated in a follow-up study conducted at S.Africa. Resting concentrations of plasma glutamine decreased at altitude ($P < 0.001$vs pre-altitude mean) and it was interesting to note that the two most elite members of the altitude-group (Commonwealth Games medallists) experienced the greatest decrease. Owing to the importance of glutamine as a substrate for key cells of the immune system, in particular lymphocytes and macrophages, (Newsholme and Castell, 1997) it is suggested that the additive stress of hypoxia decreased concentrations below a physiological concentration (which is at present undetermined), thus increasing the host's susceptibility to contracting "opportunistic infections". Several mechanisms may be implicated in the decrease in plasma glutamine concentration at altitude and a model has been proposed in an attempt to further the understanding of glutamine kinetics, exercise and infectious illness at altitude (Chapter 5).

6.4.2 Physiological Implications of Exercise at Altitude

It is clear from the preceding discussion that physiological acclimatisation to environmental hypoxia invoked a series of metabolic and cardiovascular adaptations that may potentially be implicated in the modulation of exercise performance at altitude. Due to a dearth of research conducted at moderate altitudes (1,500 m to 2,000 m) in elite athletes, the implications of 3 weeks exposure to moderate hypobaric hypoxia for maximal and supramaximal exercise performance were investigated.

An unchanged peak whole blood lactate concentration during and following recovery from exhaustive maximal and supramaximal exercise at altitude suggested that maximal anaerobic glycolytic flux was unaffected by the hypoxic stimulus (~P_IO_2 of 122 to 125 mmHg). In contrast, chronic hypoxia invoked significant changes in the cardiovascular response to exercise. Maximal heart rate decreased by 12 b.min^{-1} ($P < 0.01$ vs pre-altitude mean) possibly due to a decrease in maximal power output and/or as a physiological mechanism which serves to optimise the ventilation:perfusion ($\dot{V}_A/\dot{Q}_C$) characteristics of the lung (Chapter 5). A 13% decrease in maximal oxygen uptake ($\dot{V}O_{2max}$) was observed at altitude ($P < 0.05$ vs pre-altitude mean) despite a significant increase in minute ventilation ($P < 0.01$

vs pre-altitude mean). In combination with a maximal arterial oxygen saturation (SaO_2) of 82 ± 5%, these data would suggest that the decrease in $\dot{V}O_{2max}$ and maximal power output were mediated predominantly by an alveolar-end-capillary diffusion limitation. The reduction in $\dot{V}O_{2max}$ was significantly greater than that observed by investigators who have studied lesser-trained subjects at a similar altitude. For example, Squires and Buskirk (1982) investigated 12 sedentary subjects at sea-level and on acute exposure to altitude and demonstrated only a 7% decrease in $\dot{V}O_{2max}$ at 1,524 m. Thus, it would appear that environmental hypoxia imposes a *greater* physiological challenge, in particular for gas exchange kinetics of the elite performer. Whilst only a speculation, if future research supports the present contention of hypoxia-mediated immunosupression, then the threshold hypoxic stimulus required to activate adverse changes in immune function may be lower in the more highly trained athlete. The consistent underperception of exercise intensity noted in the present research may further increase the elite athlete's susceptibility to immunosuppression at altitude.

6.4.3 Physiological Responses During Exercise Following Return to Sea-Level

The effects of hypoxic training on sea-level exercise performance remains a controversial topic due to the large number of poorly controlled studies (Wolski et al 1996). In light of these observations, two separate studies were conducted in an attempt to elucidate the implications of altitude training on physiological indices of submaximal, maximal and supramaximal exercise performance following return to sea-level.

Submaximal exercise (Chapter 4): This chapter documented the effects of 4 weeks of altitude training at 1,500 to 2,000 m on submaximal indices of exercise performance determined three weeks following return to sea-level. Chronic hypoxia appeared to influence the metabolic response to exercise at sea-level.. A decreased lactacidosis was observed during laboratory-based *submaximal* exercise in the altitude-trained group only (Chapter 4). This was observed despite no apparent changes in CaO_2. Thus, other unquantified central or peripheral adaptations were implicated in either an increased rate of lactate clearance and/or a decreased rate of lactate efflux from skeletal muscle. However, the fact that the lactate threshold and other cardiorespiratory determinants of "running economy" were unchanged following return to sea-level suggested that 4 weeks of moderate altitude training did not confer any additional physiological advantage to that invoked by a similar programme of sea-level training. On the contrary, forty percent of the

altitude-trained subjects were unable to perform the exercise tests at sea-level as a result of either injury and/or illness incurred during the altitude sojourn. The metabolic and cardiorespiratory responses to submaximal exercise were negatively affected in one male subject who appeared to have been infected with the Epstein-Barr virus during the altitude sojourn and subsequently diagnosed with infectious mononucleosis following return to sea-level. These data highlighted the *potentially* adverse immunomodulatory effects of chronic hypoxia and the potential implications for the health and fitness of the elite subject.

Maximal exercise (Chapter 5): This chapter documented the effects of 4 weeks of altitude training at 1,640 m on the physiological response during and following recovery from maximal exercise at sea-level. Previous research has suggested that the timing of physiological testing following return to sea-level is a potentially important factor that may influence exercise performance (Suslov, 1994). Therefore, the present study incorporated physiological tests which were conducted following both 10 and 20 days return to sea-level.

Altitude training did not invoke any changes in maximal oxygen uptake ($\dot{V}O_{2max}$) or maximal running time to volitional exhaustion following either 10 or 20 days return to sea-level. Other metabolic and cardiorespiratory indices measured both during and following recovery from maximal exercise were also unaffected by chronic hypoxia.

Supramaximal exercise (Chapters 4 and 5): Previous research has failed to incorporate a performance-specific task in the experimental design which should theoretically compliment laboratory-based findings. Therefore, two separate studies investigated the physiological responses to supramaximal exercise during a standardised track session which consisted of 3 to 4 repetitions of 1000 m separated by a 2 minute walk recovery.

The metabolic and cardiovascular responses during and following recovery from supramaximal exercise at sea-level were not altered by chronic hypoxia. In contrast, group mean running velocity was significantly decreased ($P < 0.05$ vs pre-altitude mean) 3 weeks following return to sea-level in the altitude-trained group only. A decrease in absolute training intensity and/or the performance-debilitating effects of subclinical infections mediated by by the immunosupressive effects of environmental hypoxia may have been implicated in the deterioration of supramaximal exercise performance following return to sea-level.

In summary, the present research findings suggest that the elite athlete who trains at altitude is more susceptible to physical injury and infectious illness which has potentially adverse implications for exercise performance following return to sea-level. Future research should therefore focus on the potentially adverse effects of hypoxic training and address the subsequent implications for the health and fitness of the elite performer. Despite the findings outlined by the present research, it is anticipated that elite athletes will continue to incorporate hypoxic training in their exercise programmes in an attempt to potentiate sea-level performance. Thus, a series of altitude-training guidelines for the elite athlete have been formulated in an attempt to minimise the potentially adverse effects and maximise the beneficial aspects of the acclimatisation process. These are outlined in Table 6.1.

Table 6.1 Altitude Training Guidelines

Strategy	Physiological Rationale
[1] Check iron stores 3 weeks prior to the altitude sojourn. If serum ferritin concentration is > 40μg.L^{-1} start oral supplementation (200 to 300 mg daily). If < 40μg.L^{-1} consider parenteral treatment (100 mg/w). Continue iron supplementation during the altitude sojourn. Beware of constipation.	Optimise haematological adaptation
[2] Supplement with Vitamin C (0.5-1g. day^{-1}) and Vitamin E (100-500 mg; 3 times daily) and ensure an adequate intake of polyunsaturated fatty acids	Increase gastrointestinal iron uptake / Minimise haemolysis and free radical-mediated tissue damage
[3] Avoid sunburn at altitude due to increased ultraviolet radiation intensity	Minimise free-radical mediated oxidative stress / Maintain thermoregulatory function
[4] Ensure adequate fluid intake and monitor hydration status regularly (e.g. via body mass or urine volume/colour)	Prevent dehydration
[5] Ensure adequate carbohydrate intake (>8 g.kg.$^{-1}$day^{-1})	Maintain glycogen stores due to increased glycolytic flux mediated by ↑β-adrenergic activity (in particular during the first days)
[6] Ensure adequate protein intake (1.2-1.8 g.kg.$^{-1}$day^{-1}) / Administration of either BCAA (20 g.day^{-1}) or L-glutamine (5g.day^{-1})	Prevent loss of skeletal muscle mass Minimise "central fatigue" phenomenon "Boost" immunoreactivity
[7] Avoid "communal living" during a training camp (ensure separate eating/living quarters)	Minimise the spread of infectious illness
[8] Avoid maximal exercise during the first 5 days of altitude exposure. *Do not* attempt to train at the same absolute intensity as that performed at sea-level	Prevent "overtraining" response
[9] Decrease running distance if the altitude terrain is radically different to that at sea-level	Minimise the risks of physical injury

6.5 CONCLUSIONS

The experimental aims that were formulated in Chapter 2 have been realised and the null hypotheses tested by a series of investigations that were designed to establish quality control. Two separate altitude training studies have quantified the effects of chronic hypobaric hypoxia on physiological indices of submaximal, maximal and supramaximal exercise performance at altitude and following return to sea-level. This research was characterised by a high drop-out rate due to an increased incidence of injury and/or illness contracted during the altitude sojourns. Therefore, it is suggested that investigators should allow for a 60% drop-out rate if future research into hypoxia is to be conducted successfully using elite athletes.

Chronic hypobaric hypoxia (~P_IO_2 of 122 to 125 mmHg) invoked significant decreases in *maximal* oxygen uptake ($\dot{V}O_{2max}$), maximal heart rate, maximal power output despite significant increases in minute ventilation. These physiological responses were most likely mediated by an alveolar-end-capillary diffusion limitation mediated by a 13% reduction in the P_IO_2. A significantly reduced lactacidosis during an incremental submaximal treadmill test was observed in the altitude-trained group within 3 weeks of return to sea-level. However, this metabolic adaptation was not considered as physiologically significant and the implications for exercise performance were minimal considering an unchanged lactate threshold. Other cardiorespiratory markers of "running economy" remained stable following return to sea-level. There were no physiological changes during and following recovery from maximal exercise either following 10 or 20 days return to sea-level. In contrast, supramaximal exercise performance was adversely affected following 3 weeks return to sea-level in the altitude-trained group as noted by a decrease in mean running velocity.

The physiological mechanisms responsible for the modulation of exercise performance were to some extent elucidated by the present research. Resting arterial oxygen content (CaO_2) did not change either at altitude or following return to sea-level. Depressed iron stores and/or an insufficient hypoxic stimulus may have been implicated in the general lack of haematological adaptation observed at 1,500 to 2,000 m. In contrast, there was evidence to suggest that chronic hypoxia invoked adverse changes in immune function. Resting concentrations of plasma glutamine and neutrophil content were significantly decreased by

the 19th day at 1,640 m. The immunosupressive effects of hypoxia may have been implicated in the marked increase in the incidence of infectious illness that was observed at altitude.

Thus, the present research findings would suggest that four weeks of moderate altitude training at 1,500 m to 2,000 m did not improve physiological performance during and following recovery from either submaximal or maximal exercise. Chronic hypoxia appeared to have a negative effect on supramaximal exercise performance following return to sea-level. The implications of a depressed immune function for the health of the elite competitor who wishes to train at altitude requires further investigation.

CHAPTER 7

REFERENCES

Adams, W.C. Bernauer, E.M. Dill, D.B. and Bomar, Jr J.B. (1975), Effects of equivalent sea-level and altitude training on $\dot{V}O_2$ Max and running performance. *Journal of Applied Physiology* 39 (2), 262-266.

Adolfsson, S. and Ahren, K. (1971), Control mechanisms for the synthesis of glycogen in striated muscle. In, Pernow, B. and Saltin, B. (eds). *Muscle metabolism during exercise*. New York: Plenum Press, pp 257-272.

Alexander, J.K. and Grover, R.F. (1983), Mechanism of reduced cardiac stroke volume at high altitude. *Clinical Cardiology* 6, 301-303.

Altman, D.G. (1980), Statistics and ethics in medical research. How large a sample? *British Medical Journal* 281 (15), 1336-1338.

Altman, D.G. (1991), *Practical Statistics For Medical Research*. London: Chapman and Hall, pp 171-173.

Anand, I.S., Chandrashekhar, Y. and Rao, S.K. (1993), Body fluid compartments, renal blood flow and hormones at 6000m in normal subjects. *Journal of Applied Physiology* 74, 1234-1239.

Andersen, K.L. Shephard, R.J. Denolin, H. Varnaukas, E. and Masironi, R. (1971), Fundamentals of exercise testing. Geneva. World Health Organisation, pp 58-73.

Ardawi, M.S.M. (1983), Glutamine metabolism in lymphocytes of the rat. *Biochemistry Journal* 212, 835-842.

Ardawi, M.S.M. and Newsholme, E.A. (1985), Metabolism in lymphocytes and its importance to the immune response. *Essays in Biochemistry* 21, 1-44.

Ardawi, M.S.M. (1988), Glutamine and glucose metabolism in human peripheral lymphocytes. *Metabolism* 37, 99.

Ardawi, M.S.M. and Jamal, Y.S. (1990), Glutamine metabolism in skeletal muscle of glucocorticoid -treated rats. *Clinical Science* 79, 139-147.

Armstrong. L.E. Maresh, C.M. Castellani, J.W. Bergeron, M.F. Kenefick, R.W. LaGasse, K.E. and Riebe, D. (1994), Urinary indices of hydration status. *International Journal of Sports Nutrition* 4 (3), 265-279.

Asahina, K. Ikai, M. Agawas, Y. and Kuroda, Y. (1966), A study on acclimatization to altitude in Japanese athletes. *Schweizerishe Zeitschrift Fuer Sportmedizin* 14, 240-245.

Asano, K. Sub, S. Matsuzaka, A. Hirakoba, K. Nagai J. and Kawaoka, T. (1986), The influences of simulated high altitude training on work capacity and performance in middle and long distance runners. *Bulletin of Institute of Health and Sports Sciences* 9, 195-202.

Asano, K. Mazzeo, R.S. McCullough, R.E. Wolfel, E.E. and Reeves, J.T. (1997), Relation of sympathetic activation to ventilation in man at 4,300 m altitude. *Aviation, Space and Environmental Medicine* 68, 104-110.

Asimov, I. (1984), *Asimov's New Guide To Science. A Revised Edition.* London: Penguin group.

Åstrand, P.O. (1994), Introduction-Man as an athlete, in Harries, M. Williams, C. Stanish, W. and Micheli, L. (eds). *Oxford Textbook of Sports Medicine*, London: Oxford University Press, London, pp 1-10.

Atrial natriuretic peptide (editorial). (1986), Lancet 2, 371-372.

Aizenman, E. Hartnett, K.A. and Reynolds, I.J. (1990), Oxygen free radicals regulate NMDA receptor function via a redox modulatory site. *Neuron* 5, 841-846.

Babij, P. Matthews, S.M. and Rennie, M.J. (1983), Changes in blood ammonia, lactate and amino acids in relation to workload during bicycle ergometer exercise in man. *European Journal of Applied Physiology and Occupational Physiology* 50, 405-411.

Bachorik, P.S. (1982), Collection of blood samples for lipoprotein analysis. *Clinical Chemistry* 28 (6), 1375-1378.

Bailey, D.M. Davies, B. and Gandy, G. (1996), Serum urea and delta heart rate as markers of exercise intensity in National standard distance runners training at moderate altitude (1500-2000 metres). (Abstract). In: European College of Sport Science, First Annual Congress, Frontiers in Sport Science, the European Perspective, Marconnet, P. Gaulard, J. Margaritis, I. and Tessier, F (eds) 1, 594-595.

Bailey, D.M. Davies, B. Romer, L. and Gandy, G. (1996), Physiological implications of moderate altitude training (1640 metres) on sea-level endurance performance in elite distance runners. *British Journal of Sports Medicine*. Abstract 30, pp 370.

Bailey, D.M. Davies, B. Romer, L. and Gandy, G. (1996), The effects of moderate altitude training on serum urea and delta heart rate in an elite cohort of distance runners (Abstract). *British Journal of Sports Medicine* 31 (1), 89.

Bailey, D.M. and Davies, B. (1997), Physiological implications of altitude training for endurance performance at sea-level - A review. *British Journal of Sports Medicine* 31, 183-190.

Bailey, D.M. and Davies, B. (1997), Recovery from infectious mononucleosis following altitude training in an elite middle distance runner. *British Journal of Sports Medicine* 31, 153-154.

Baker, M.A. Davis, P.A. and Hawkins, M.J. (1983), Effect of dehydration and hyperosmolality on thermoregulatory water losses in exercising dogs. *American Journal of Physiology* 244 (*Regulatory Integrative Comparative Physiology*), R516-R521.

Balke, B. Nagle, F.J. and Daniels, J. (1965), Altitude and maximum performance in work and sports activity. *Journal of the American Medical Association* 194 (6), 646-649.

Balke, B. and Snow, C. (1969), Anthropological and physiological observations on Tarahumara endurance runners. *American Journal of Physical Anthropology* 23, 293-302.

Ball, M. and Mann, J. (1994), Lipids and Heart Disease. A Guide for the Primary Care Team. Second Edition. London: Oxford University Press.

Banister, E.W. and Woo, W. (1978), Effects of simulated altitude training on aerobic and anaerobic power. *European Journal of Applied Physiology and Occupational Physiology* 38, 55-69.

Banister, E.W. Rajendra, W. and Mutch, B.J.C. (1985), Ammonia as an indicator of exercise stress: implications of recent findings to sports medicine. *Sports Medicine* 2 (1), 34-46.

Barron, G.L. Noakes, T.D. Levy, W. Smith, C. and Millar, R.P. (1985), Hypothalamic dysfunction in overtrained athletes. *Journal of Clinical Endocrinology and Metabolism* 60, 803-806.

Bason, R. and Billings, C.E. (1969), Effects of high altitude on lipid components of human serum. *Archives of Environmental Health* 19, 183-185.

Bauer, C. and Kurtz, A. (1989), Oxygen sensing in the kidney and its relation to erythropoietin production. *Annual Reviews in Physiology* 51, 845-856.

Baxter, C. and Reilly, T. (1983), Influence of time of day on all-out swimming. *British Journal of Sports Medicine* 17, 122-127.

Beall, C.M. Blangero, J. Williams-Blangero, S. and Goldstein, M.C. (1994), Major gene for percent of oxygen saturation of arterial hemoglobin in Tibetan highlanders. *American Journal of Physical Anthropology* 95, 271 - 276.

Bedu, M. Fellmann, N. Spielvogel, H. Falgairette, G. van Praagh, E. and Coudert, J. (1991), Force velocity and 30-s Wingate tests in boys at high and low altitudes. *Journal of Applied Physiology* 70 (3), 1031-1037.

Bedu, M. Falgairette, G. Van Praagh, E. and Coudert, J. (1994), Effect of chronic hypoxia and socioeconomic status on anaerobic power of 10 to 12 year old Bolivian boys. *International Journal of Sports Medicine* 15 (Supplement 2), S84-S89.

Beidleman, BA. Muza, S.R. Fulco, C.S. Rock, P.B. Lyons, T. Hoyt, R.W. and Cymerman, A. (1994), Decreased lactate accumulation following acclimatization to 4300 M is retained after eight days at sea level. *Medicine and Science in Sports and Exercise* 26, Supplement 5, Abstract 118

Bender, P.R. Groves, B.M. McCullough, R.E. McCullough, R.G. Huang, S.Y. Hamilton, A.J. and Wagner, P.D. (1988), Oxygen transport to exercising leg in chronic hypoxia. *Journal of Applied Physiology* 65, 2592-2597.

Bender, P.R. Groves, B.M. McCullough, R.E. McCullough, R.G. Trad, L. Young, A.J. Cymerman A. and Reeves, J.T. (1989), Decreased exercise muscle lactate release after high altitude acclimatization. *Journal of Applied Physiology* 67 (4), 1456-1462.

Benoit, H. Costes, F. Castells, J. Busso, T. Denis, C. Lacour, J.R. and Geyssant, A. (1992), Endurance training in normobaric hypoxia: ventilatory response and ability to exercise *International Journal of Sports Medicine* 13 (90), Abstract.

Bergh, U. and Ekblom, B. (1980), Influence of muscle temperature on maximal muscle strength and power output in human skeletal muscles. *Acta Physiologica Scandinavica* 107, 33-37.

Berglund, B. and Hemmingsson, P. (1987), Effect of re-infusion of autologous blood on exercise performance in cross-country skiers. *International Journal of Sports Medicine* 8, 231-233.

Berglund, B. (1992), High-altitude training. Aspects of haematological adaptation. *Sports Medicine* 14 (5), 289-303.

Berglund, B. Fleck, S.J. Kearney, J.T. and Wide, L. (1992), Serum erythropoietin in athletes at moderate altitudes. *Scandinavian Journal of Medicine and Science in Sports* 2, 21-25.

Bergstrom, J. Furst, P. and Noree, L-O (1974) Intracellular free amino acid concentration in human muscle tissue. *Journal of Applied Physiology* 36, 693 -697.

Bernt, E. and Bergmeyer, H.U. (1974), L-glutamate, u-v assay with GDH and NAD, in Bergmeyer, H.U. (ed). *Methods of Enzymatic Analysis*, London: Academic Press, pp 1704-1708.

Bert, P. (1943), Barometric Pressure: Researches in Experimental Physiology. Translated by M.A. Hitchcock and R.A. Hitchcock. College Book Co, Columbus, Ohio.

Bigard, X. Satabin, P. Leger, C. Louisy, F. and Guezennec, C. (1991), Effects of polyunsaturated fatty acids on physical performance at high altitude. Proceedings from Second IOC World Congress on Sports Sciences, Barcelona, pp 260-261.

Bigard, A.X. Brunet, A. Guezennec, C.Y. and Monod, H. (1991), Skeletal muscle changes after endurance training at high altitude. *Journal of Applied Physiology* 71, 2114-2121.

Bigard, A.X. Satabin, P. Lavier, P. Canon, F. Taillandier, D. and Guezennec, C.Y. (1993), Effect of protein supplementation during prolonged exercise at moderate altitude on performance and plasma amino acid pattern. *European Journal of Applied Physiology and Occupational Physiology* 66, 5-10.

Bigard, A.X. Lavier, P. Ullmann, L. Legrand, H. Douce, P. and Guezennec, C.Y. (1996), Branched-chain amino acid supplementation during repeated prolonged skiing exercises at altitude. *International Journal of Sport Nutrition* 6, 295-306.

Bigland-Ritchie, B. and Vollestad, N.K. (1988), Hypoxia and fatigue: how are they related? In Sutton, J.R. Houston, C.S. and Coates, G. (eds), *Hypoxia: The tolerable limits*. Indianapolis: Benchmark, pp 315-325.

Birgegard, G. Hogman, C. Killander, A. Levander, H. and Simonson, B. (1977), Serum ferritin and erythrocyte 2,3-DPG during quantified phlebotomy and iron treatment. *Scandinavian Journal of Haematology* 19 (4), 327-333.

Biselli, R. Pecci, G. Oliva, C. Fattorossi, A. D'Amelio, R. and Barsotti, P. (1992), Effects of hypobaric hypoxia (HH) on polymorphonuclear granulocytes (PMNL) respiratory

activity in HH-acclimatized and non-acclimatized rats. *International Journal of Sports Medicine* 13, Abstract.

Bjuerstrom, R.L. and Schoene, R.B. (1986), Ventilatory control in elite synchronized swimmers. *American Review of Respiratory Diseases* 133 (Supplement), A134.

Blair, S.N. Ellsworth, N.M. Haskell, W.H. Stern, M.P. Farquhar, J.W. and Wood, P.D. (1981), Comparison of nutrient intake in middle-aged men and women runners and controls. *Medicine and Science in Sports and Exercise* 13, 310-315.

Blonc, S. Falgairette, G. Bedu, M. Fellmann, N. Spielvogel, H. and Coudert, J. (1994), The effect of acute hypoxia at low altitude and acute normoxia at high altitude on performance during a 30-s Wingate test in children. *International Journal of Sports Medicine* 15 (7), 403-407.

Blume, F.D. Boyer S.J. Braverman, L.E. Cohen, A., Dirkse, J. and Mordes, J.P. (1984), Impaired osmoregulation at high altitude. *Journal of the American Medical Association* 252, 524-526.

Borg, G.A.V. (1973), Perceived exertion: a note on history and methods. *Medicine and Science in Sports and Exercise* 5 (2), 90-93.

Boutellier, U. Marconi, C. diPrampero, P.E. and Cerretelli, P. (1982), Effects of chronic hypoxia on maximal performance. *Clinical Respiratory Physiology* 18 (Supplement 4) 39-44.

Boutellier, U. Deriaz, O. diPrampero, P.E. and Cerretelli, P. (1990), Aerobic performance at altitude: Effects of acclimatization and haematocrit with reference to training, *International Journal of Sports Medicine* 11 (Supplement 1), S21-S26.

Braun, B. Butterfield, G.E. Mawson, J.T. Muza, S. Dominick, B.S. Rock, P.B. and Moore, L.G. (1997), Women at altitude: Substrate oxidation during steady-state exercise at sea level and after acclimatization to 4300 meters elevation. *Medicine and Science in Sports and Exercise* Supplement 136, Abstract 784.

Brien, A.J. and Simon, T.L. (1987), The effect of red blood cell reinfusion on 10-km race time. *Journal of the American Medical Association* 257, 2761-2765.

Brien, A.J. Harris, R.J. and Simon, T.L. (1989), The effects of an autologous infusion of 400 ml red blood cells on selected hematological parameters and 1,500 m race time in highly trained runners. *Bahrain Medical Bulletin* 11, 6-16.

Broberg, S. and Sahlin, K. (1989), Adenine nucleotide degradation in human skeletal muscle during prolonged exercise. *Journal of Applied Physiology* 67, 116-122.

Brodde, E.D. Daul, A. and O'Hara, N. (1984), β-adrenoreceptor changes in human lymphocytes, induced by dynamic exercise. *Naunyn Schmiedebergs Arch Pharmacol* 325, 190-192.

Brooks, G.A. Butterfield, G.E. Wolfel, R.R. Groves, B.M. Mazzeo, R.S. Sutton, J.R. Wolfel, E.E. and Reeves, J.T. (1991), Decreased reliance on lactate during exercise after acclimatisation to 4,300 m. *Journal of Applied Physiology* 71, 333-341.

Brooks, G.A. Wolfel, E.E. Groves, B.M. Bender, P.R. and Butterfield, G.E. (1992), Muscle accounts for glucose disposal but not blood lactate appearance during exercise after acclimatization to 4300 m. *Journal of Applied Physiology* 72, 2435-2445.

Brooks, G.A. Roberts, A.C. Butterfield, G.E. Wolfel, E.E. and Reeves, J.T. (1994), Altitude exposure increases reliance on glucose. *Medicine and Science in Sports and Exercise* 26 (Supplement 5), Abstract 120.

Brooks-Gunn, J. Gargiulo, J.M. and Warren, M.P. (1986), The effect of cycle phase on the adolescent swimmers. *Physician and Sportsmedicine* 14 (3), 182-192.

Brouns, F. Beckers, E. Wagemakers, A.J.M. and Saris, W.H.M. (1990), Ammonia accumulation during highly intensive long-lasting cycling: Individual observations. *International Journal of Sports Medicine* 11, S78-S84.

Brouns, F. (1992), Nutritional aspects of health and performance at lowland and altitude. *International Journal of Sports Medicine* 13 (Supplement 1), S100-S106.

Brown, M.S. and Goldstein, J.L. (1990), Pharamacological Basis of Therapeutics, in Gillman, D. (ed), (1990), New York: Pergamon Press, pp 874.

Buick, F.J. Gledhill, N. Froese, A.B. Spriet, L. and Meyers, E.C. (1980), Effect of induced erythrocythemia on aerobic work capacity. *Journal of Applied Physiology* 48, 636-642.

Buskirk, E.R. Kollias, J. Akers, R.F. Prokop, E.K. and Reategui, E.P. (1967), Maximal performance at altitude and on return from altitude in conditioned runners. *Journal of Applied Physiology* 23 (2), 259-266.

Buskirk, E.R. (1996), Historical perspectives regarding our understanding of physical performance during hypoxia. *Research Quarterly for Exercise and Sport* 67 (Supplement 3), 73-75.

Butterfield, G.E. Gates, J. Flemming, S. Brooks, G.A. and Sutton, J.R. (1992), Increased energy intake minimizes weight loss in men at high altitude. *Journal of Applied Physiology* 72, 1741-1748.

Buttner, J. Borth, R. Boutwell, J. Broughton, P.M.G. and Bowyer, R.C. (1979), Approved recommendation (1978) on quality control in clinical chemistry. Part 1. General principles and terminology. *Clinical Chim Acta* 98, 129-143F.

Bye, P.T.P. Esau, S.A. Walley, K.R. Macklem, P.T. and Pardy, R.L. (1984), Ventilatory muscles during exercise in air and oxygen in normal men. *Journal of Applied Physiology* 56, 464-471.

Byrne-Quinn, E. Weil, J.V. and Sodal, I.E. (1971), Ventilatory control in the athlete. *Journal of Applied Physiology* 30, 91-98.

Carraro, F. Kimbrough, T.D. and Wolfe, R.R. (1993), Urea kinetics in humans at two levels of exercise intensity. *Journal of Applied Physiology* 75 (3), 1180-1185.

Castell, L.M. Poortmans, J.R. and Newsholme, E.A. (1996), Does glutamine have a role in reducing infections in athletes? *European Journal of Applied Physiology and Occupational Physiology* 73, 488-490.

Celsing, F. Svedenhag, J. Pilhstedt, P. and Ekblom, B. (1987), Effects of anaemia and stepwise-induced polycythemia on maximal aerobic power in individuals with high and low haemoglobin concentrations. *Acta Physiological Scandinavica* 31, 47-54.

Cerretelli, P. DiPrampero, P.E. and Rennie, D.W. (1970), Measurement of mixed venous oxygen tension by a modified rebreathing technique. *Journal of Applied Physiology* 28, 707-711.

Cerretelli, P. and DiPrampero, P.E. (1985), Aerobic and anaerobic metabolism during exercise at altitude, in Rivolier, J. Cerretelli, P. Foray, J. and Segantini, P. (eds). *High altitude deterioration*, Basel: Karger, pp 1-19.

Cheng, B. Kuipers, H. Snyder, A.C. Keizer, H.A. Jeukendrup, A. and Hesselink, M. (1992), A new approach for the determination of ventilatory and lactate thresholds. International *Journal of Sports Medicine* 13 (7), 518-522.

Chohan, I.S. Sing, I. Balakrishnan, K. and Talwar, G.P. (1975), Immune responses in human subjects at high altitude. *International Journal of Biometeorology* 19, 137-143.

Christensen, E.H. and Forbes, W.H. (1937), Der kreislauf in grossen höhen. *Skandinavisches Archives für Physiologie* 76, 75-89.

Clark, M.R. (1988), Senescence of red blood cells: problems and progress. *Physiological Reviews* 68, 503-553.

Clarkson, P.M. (1990), Tired blood: Iron deficiency in athletes and effects of iron supplementation. *Sports Science Exchange* 3 (28).

Clement, D.B. and Asmundson, R.C. (1982), Nutritional intake and hematological parameters in endurance runners. *Physician and Sportsmedicine* 10, 37-43.

Connett, R.J. Honig, C.R. Gayeski, E.J. and Brooks, G.A. (1990), Defining hypoxia: a systems view of $\dot{V}O_2$, glycolysis, energetics, and intracellular PO_2. *Journal of Applied Physiology* 68 (3), 833-842.

Cook, J.D. Skikne, B.S. and Baynes, R.D. (1993), Serum transferrin receptor. *Annual Reviews in Medicine* 44, 63-74.

Cook, J.D. (1994), The effect of endurance training on iron metabolism. *Seminars in Haematolgy* 31, 146-154.

Cooper, D.M. Wasserman, D.H. Vranic, M. and Wasserman, K. (1986), Glucose turnover in response to exercise during high- and low-FIO_2 breathing in man. *American Journal of Physiology* 251 (*Endocrinology and Metabolism* 14), E209-E214.

Costill, D.L. and Sparks, K.E. (1973), Rapid fluid replacement following thermal dehydration. *Journal of Applied Physiology* 34, 299-303.

Costongs, G.M.P.J. Janson, P.C.W. Bas, B.M. Hermans, J. Van Wersh, J.W.J. and Brombacher, P.J. (1985), Short and long term intra-individual variations and critical differences of clinical laboratory parameters. *Journal of Clinical Chemistry and Clinical Biochemistry* 23, 7-16.

Coudert, J. (1992), Anaerobic performance at altitude. *International Journal of Sports Medicine* 13 (Supplement 1), S82-S85.

Cunningham, D.A. and Magel, J.R. (1970), The effect of moderate altitude on post exercise blood lactate. *Internationale Zeitschrift fur Angewandte Physiologie Einschliesslich Arbeitsphysiologie* 29 (1), 94-100.

Curran-Everett, L. Zhuang, J.T. Droma, T.S. and Moore, L.G. (1992), Work efficiency and altitude of origin in Tibet. *American Journal of Physical Anthropology* 14. (Supplement 66). Abstract.

Czajkowski, W. (1982), A simple method to control fatigue in endurance training, in Komi, P.V. (ed) *Experimental and Sports Biology*, Champaign, Illinois: Human Kinetics pp 207-212.

Dacie, J.V. and Lewis, S.M. (1968), *Practical Haematology. 4th Edition*, London: Churchill, pp 45-49.

Dallman, P.R. (1993), Biochemical basis for the manifestations of iron deficiency. Annual *Reviews in Nutrition* 6, 13-40.

Damian, A.C. and Pitts, R.F. (1970), Rates of glutaminase I and glutamine synthetase reactions in rat kidney *in vivo*. *American Journal of Physiology* 218, 1249-1255.

Daniels, J. and Oldridge, N. (1970), The effects of alternate exposure to altitude and sea - level on world-class middle-distance runners. *Medicine and Science in Sports and Exercise* 2 (3), 107-112.

Davies, C.T. and Sargeant, A.J. (1974), Effects of hypoxic training on normoxic maximal aerobic power output. *Journal of Applied Physiology* 33 (3), 227-236.

Davies, K.J.A. Quintanilha, A.T. Brooks, G.A. and Packer, L. (1982), Free radicals and tissue damage produced by exercise. *Biochemistry and Biophysics Research Communications* 107, 1198-1205.

Décombaz, J. Reinhardt, P. and Anantharaman, K. (1979), Biochemical changes in a 100km run: free amino acids, urea and creatinine. *European Journal of Applied Physiology and Occupational Physiology* 41, 61-72.

DeGraff, A.C., Grover, R.F. and Johnson, R.L. (1970) Diffusing capacity of the lung in Caucasians native to 3100 m. Journal of Applied Physiology. Volume 29, 71-76.

De Mendoza, S. Nucete, H. Ineichen, E. Salazar, E. Zerpa, A. and Glueck, C.J. (1979), Lipids and lipoproteins in subjects at 1000 and 3500 meter altitudes. *Archives of Environmental Health* 5, 308-311.

Dempsey, J.A. Reddan, W.G. and Birnbaum, M.L. (1971), Effects of acute through life-long hypoxic exposure on exercise pulmonary gas exchange. *Respiratory Physiology* 13, 62-89.

Dempsey, J.A. Hanson, P. and Henderson, K. (1984), Exercise-induced alveolar hypoxemia in healthy human subjects at sea level. *Journal of Physiology London* 355, 161-175.

Dempsey, J.A. (1986) J.B. Wolffe memorial lecture. Is the lung built for exercise? *Medicine and Science in Sports and Exercise* 18 (2), 143-155.

Denell, R.F. Rendell, H.M. and Hailwood, E. (1988), Late pliocene artifacts from Northern Pakistan. *Current Anthropology* 29, 495-498.

Desauliniers, N. Moerland, T.S. and Sidell, B.D. (1996), High lipid content enhances the rate of oxygen diffusion through fish skeletal muscle. *American Journal of Physiology* 271, R42-R47.

Desplanches, D. Hoppeler, H. Linoissier, M.T. Denis, C. Claassen, H. Dormois, D. Lacour, J.R. and Geyssant, A. (1993), Effects of training in normoxia and normobaric hypoxia on human muscle ultrastructure. *European Journal of Applied Physiology and Occupational Physiology* 425, 263-267.

Dickerson, R.E. (1978), Chemical evolution and the origin of life. *Scientific American* 239 (3), 62-78.

Diess, A. (1983) Iron metabolism in reticuloendothelial cells. *Seminars in Haematology* 20, 81-90.

Dill, D.B. and Adams, W.C. (1971), Maximal oxygen uptake at sea-level and at 3,090-m altitude in high school champion runners. *Journal of Applied Physiology* 30 (6), 854-859.

Dill, D.B. Braithwaite, K. Adams, W.C. and Bernauer, E.M. (1974), Blood volume of middle distance runners: effect of 2300 m altitude and comparison with non-athletes. *Medicine and Science in Sports and Exercise* 6, 1-7.

Dill, D.B. and Costill, D.L. (1974), Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *Journal of Applied Physiology* 37 (2), 247-248.

Di Prampero, P.M. Mognoni, P. and Veicsteinas, A. (1982), The effects of hypoxia on maximal anaerobic alactic power in man, in Brendel, D. and Zink, J. (eds). *High altitude physiology and medicine*. New York: Springer, pp 88-93.

Dick, F.W. (1992), Training at altitude in practice. *International Journal of Sports Medicine*. 13, S203-S205.

Dolny, D.G. and Lemon, P.W.R. (1988), Effect of ambient temperature on protein breakdown during prolonged exercise. *Journal of Applied Physiology* 64 (2), 550-555.

Dugal, R. and Bertrand, M. (1976), Doping, in IOC Medical Commission Handbook. Montreal: Commité Organisateur des Jeux Olympiques, pp 1-31.

Durnin, J.V.G.A. and Womersley, J. (1974), Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *British Journal of Nutrition* 32, 77-97.

Eckardt, K.U. Boutellier, U. Kurtz, A. Schopen, M. and Koller, E.A. (1989), Rate of erythropoietin formation in humans in response to acute hypobaric hypoxia. *Journal of Applied Physiology* 66, 1785-1788.

Edwards, H.T (1936), Lactic acid in rest and work at high altitudes. *American Journal of Physiology* 116, 367-375.

Eichner, E.R. (1987), Infectious mononucleosis: Recognition and management in athletes. *The Physician and Sportsmedicine* 15 (12), 61-70.

Ekblom, B. and Berglund, B. (1991), Effect of erythropoietin administration on maximal aerobic power. *Scandinavian Journal of Sports Medicine* 1, 88-93.

Engfred, K. Kjaer, M. Secher, N.H. Friedman, D.B. Hanel, B. Nielsen, O.J. Bach, F.W. Galbo, H. and Levine, B.D. (1994), Hypoxia and training-induced adaptation of hormonal responses to exercise in humans. *European Journal of Applied Physiology and Occupational Physiology* 68, 303-309.

Eriksson, L.S. Broberg, S. and Bjőrkman, O. (1985), Ammonia metabolism during exercise in man. *Clinical Physiology* 5, 325-336.

Ernsting, J. and King, P. (1995), *Aviation Medicine, Second Edition.* London: Butterworth-Heinemann Ltd, pp 7-8.

Escourrou, P. Johnson, D.G. and Rowell, L.B. (1984), Hypoxemia increases plasma catecholamine concentrations in exercising humans. *Journal of Applied Physiology* 57, 1507-1511.

Euler, U.S. (1974), Sympathoadrenal activity in physical exercise. *Medicine and Science in Sports and Exercise* 3, 165-173.

Falgairette, G. Bedu, M. Fellmann, N. Spielvogel. H. Van Praagh, E. Obert, P. and Coudert, J (1994), Evaluation of physical fitness from field tests at high altitude in circumpubertal boys: comparison with laboratory data. *European Journal of Applied Physiology and Occupational Physiology* 69 (1), 36-43.

Faulkner, J.A. Daniels, J.T. and Balke, B. (1967), Effects of training at moderate altitude on physical performance capacity. *Journal of Applied Physiology* 23 (1), 85-89.

Faulkner, J.A. Kollias, J. Favour, C.B. Buskirk, E.R. and Balke, B. (1968), Maximum aerobic capacity and running performance at altitude. *Journal of Applied Physiology* 24 (5), 685-691.

Favier, R. Spielvogel, H. Desplanches, D. Ferretti, G. Kayser, B. and Hoppeler, H. (1995), Maximal exercise performance in chronic hypoxia and acute normoxia in high-altitude natives. *Journal of Applied Physiology* 78 (5), 1868-1874.

Favier, R. Spielvogel, D. Desplanches, D. Ferretti, G. Kayser B. Grunenfelder, A. Leuenberger, M. Tuscher, L. Cacares, E. and Hoppeler, H. (1995), Training in hypoxia vs. training in normoxia in high-altitude natives. Journal of Applied Physiology 78 (6), 2286-2293.

Fedders, A. and Salvadori, C. (1980), *Peoples and Cultures of Kenya. Nairobi.* Transafrica and London: Rex Collings.

Fellmann. N. Bedu, M. Spielvogel, H. Falgairette, G. Van Praagh, E. Jarrige, J.F. and Coudert, J. (1988), Anaerobic metabolism during pubertal development at high altitude. *Journal of Applied Physiology* 64, 1382-1386.

Ferezou, J. Richalet, J.P. Coste, T. and Rathat, C. (1988), Changes in plasma lipids and lipoprotein cholesterol during a high altitude mountaineering expedition (4800 m). *European Journal of Applied Physiology and Occupational Physiology* 57, 740-745.

Ferretti, G. Hauser, H. and DiPrampero, P.E. (1990), Maximal muscular power before and after exposure to chronic hypoxia. *International Journal of Sports Medicine* 11 (Supplement 1), 531-534.

Forte, V.A. Leith, D.E. Muza, S.R. Fulco, C.S. and Cymerman, A. (1997), Ventilatory capacities at sea level and high altitude. *Aviation, Space and Environmental Medicine* 68, 488-493.

Fraser, C.G. and Fogarty, Y. (1989), Interpreting laboratory results. *British Medical Journal* 298, 165-166.

Frayn, K.N. Khan, K. and Coppack, S.W. (1991), Amino acid metabolism in human subcutaneous adipose tissue *in vivo*. *Clinical Science* 80, 471-474.

Friedewald, W.T. Levy, I. and Fredrickson, D.S. (1972), Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without the use of the preparative ultracentrifuge. *Clinical Chemistry* 18, 499-502.

Friedman, D.B. Levine, B.D. Hanel, B. Engfred, K. Clifford, P.S. and Secher, N.H. (1990), Endurance training and the hypoxic ventilatory response. *Medicine and Science in Sports and Exercise* 22, S99, Abstract 592..

Friedman, M. (1937), The use of ranks to avoid the assumption of normality implicit in analysis of variance. *Journal of the American Statistical Association* 32, 675-701.

Frisancho, A.R. Martinez, C. Velasquez, T. Sanchez, J. and Montoye, H. (1973), Influence of developmental adaptation on aerobic capacity at high altitude. *Journal of Applied Physiology* 34, 176-180.

Frisancho, A.R. Frisancho, H.G. Milotich, M. Brutsaert, T. Albalak, R. Spielvogel, H. Villena, M. Vargas, E. and Soria, R. (1995), Developmental, genetic and environmental components of aerobic capacity at altitude. *American Journal of Physical Anthropology* 96, 431-442.

Fulco, C.S. Rock, P.B. Trad, L.A. Rose, M.S. Forte, V.A. Young, P.M. and Cymerman, A. (1994), Effect of caffeine on submaximal exercise performance at altitude. *Aviation, Space and Environmental Medicine* 65, 539-545.

Fulco, C.S. and Cymerman, A. (1988), Human Performance and acute hypoxia, in Pandolf, K.B. (ed). *Human Performance Physiology and Environmental Medicine at Terrestrial Extremes*, Indianapolis: Benchmark Press, pp 467-493.

Garber, A.J. Karl, I.E. and Kipnis, D.M. (1976c), Alanine and glutamine synthesis and release from skeletal muscle. IV. beta-Adrenergic inhibition of amino acid release. *Journal of Biology and Chemistry* 251, 851-857.

Ge, R.L. Chen, Q.H. Wang L.H. Gen, D. Yang, P. Kubo, K. Fujimoto, K. Matsuzawa, Y. Yoshimura, K. Takeoka, M. and Kobayashi, T. (1994), Higher exercise performance and lower VO_2 max in Tibetan than Han residents at 4,700 m altitude. *Journal of Applied Physiology* 77, 684-691.

Giesbrecht, G.G. (1995), The respiratory system in a cold environment. *Aviation and Space Environmental Medicine* 66, 890-902.

Gledhill, N. (1985), The influence of altered blood volume and oxygen transport capacity on aerobic performance. *Exercise and Sports Science Reviews* 13, 75 - 93.

Gleser, M.A. and Vogel, J.A. (1973), Endurance capacity for prolonged exercise on the bicycle ergometer. *Journal of Applied Physiology* 34, 438-442.

Goforth, H.W. Campbell, Jr N.L. Hodgdon, J.A. and Sucec, A.A. (1982), Hematologic parameters of trained distance runners following induced erythrocythemia. *Medicine and Science in Sports and Exercise* 14, 174.

Goldspink, G. (1977), Energy cost of locomotion, in, Alexander, R.McN. and Goldspink, G. (eds) *Mechanics of Energetics of Animal Locomotion*. London: Chapman and Hall, pp 153-167.

Goldstein, L. Schrock, H. and Cha, C.J. (1980), Relationship of muscle glutamine production to renal ammonia metabolism. *Biochemistry Society Transactions* 8, 509-510.

Gordon, D.J. Probstfield, J.L. and Garrison, R.J. (1989), High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation* 79, 8-15.

Gore, C.J. Hahn, A.G. Watson, D.B. Norton, K.I. Campbell, D.P. Scroop, G.S. Emonson, D.L. Wood, R.J. Ly, S.V. Bellenger, S.J. and Lawton, E.W. (1995), $\dot{V}O_2$ Max and arterial O_2 saturation at sea-level and 610 m. *Medicine and Science in Sports and Exercise* 27 (Supplement 5), Abstract 42.

Gore, C.J. Hahn, A.G. Scroop, G.C. Watson, D.B. Norton, K.I. Wood, R.J. Campbell, D.P. and Emonson, D.L. (1996), Increased arterial desaturation in trained cyclists during maximal exercise at 580 m altitude. *Journal of Applied Physiology* 80 (6), 2204-2210.

Graham, T.E. and MacLean, D.A. (1992), Ammonia and amino acid metabolism in human skeletal muscle during exercise. *Canadian Journal of Physiology and Pharmacology* 70, 132-141.

Grassi, B. Ferretti, G. Kayser, B. Marzorati, M. Colombini, A. Marconi, C. and Cerretelli, P. (1995), Maximal rate of blood lactate accumulation during exercise at altitude in humans. *Journal of Applied Physiology* 79 (1), 331-339.

Green, D.J. O'Driscoll, G. Blanksby, B.A. and Taylor, R.R. (1996), Control of skeletal muscle blood flow during dynamic exercise: Contribution of endothelium-derived nitric oxide. *Sports Medicine* 21 (2), 119-146.

Green, H.J. Sutton, J.R. Cymerman, A. Young, P.M. and Houston, C.S. (1989), Operation Everest II: adaptations in human skeletal muscle. *Journal of Applied Physiology* 66 (5), 2454-2461.

Green, H.J. Sutton, J.R. Wolfel, E.E. Reeves, J.T. Butterfield, G.E. and Brooks, G.A. (1992), Altitude acclimatization and energy metabolic adaptations in skeletal muscle during exercise. *Journal of Applied Physiology* 73 (6), 2701-2708.

Grover, R.F. Reeves, J.T. Grover, E.B. and Leathers, J.E. (1967), Muscular exercise in young men native to 3,100 m altitude. *Journal of Applied Physiology* 22 (3), 555-564.

Groves, B.M. Reeves, J.T. Sutton, J.R. Wagner, P.D. and Cymerman, A. (1987), Operation Everest II: elevated high-altitude pulmonary resistance unresponsive to oxygen. *Journal of Applied Physiology* 63 (2), 521-530.

Groves, B.M. Droma, T. Sutton, J.R. McCullough, R.G. McCullough, R.E. Zhuang, J. Rapmund, G. Sun, S. Janes, C. and Moore, L.G. (1993), Minimal hypoxic pulmonary hypertension in normal Tibetans at 3,658 m. *Journal of Applied Physiology* 74 (1), 312-318.

Hackett, P. Roach, R.C. Schoene, R.B. et al. (1988), Abnormal control of ventilation in high altitude pulmonary edema. *Journal of Applied Physiology* 64, 1268-1272.

Hackney, A.C. Coyne, J.T. Pozos, R. Feith, S. and Seale, J. (1995), Validity of urine-blood hydrational measures to assess total body water changes during mountaineering in the sub-Arctic. *Arctic Medical Research* 54 (2), 69-77.

Hahn, A.G. Telford, R.D. Tumilty, D.M. McBride, M.E. Campbell, D.P. Kovacic, J.C. Batschi, R. and Thompson, P.A. (1992), Effect of supplementary hypoxic training on physiological characteristics and ergometer performance of elite rowers. *Excel* 8, 127-138.

Haldane, J.S. and Priestley, J.G. (1935), *Respiration, second edition*, Connecticut: Yale University Press, pp 323.

Hammond, M.D. Gale, G.E. Kapitan, K.S. Ries, A. and Wagner, P.D. (1986), Pulmonary gas exchange in humans during exercise at sea level. *Journal of Applied Physiology* 60, 1590-1598.

Hannon, J.P. Shields, J.L. and Harris, C.W. (1967), High altitude acclimatization in women, in, Proceedings of international symposium on the effects of altitude on physical performance, Athletic Institute, Chicago, pp 37-44.

Hannon, J.P. Shields, J.L. and Harris, C.W. (1969), Effects of altitude acclimatization on blood composition of women. *Journal of Applied Physiology* 26, 540-547.

Hansen, J.E. Vogel, J.A. Stelter, G.P. and Consolazio, C.F. (1967), Oxygen uptake in man during exhaustive work at sea level and high altitude. *Journal of Applied Physiology* 23 (4), 511-522.

Harper, K.M. Stray-Gunderson, J. Schecter, M.B. and Levine, B.D. (1994), Training at moderate altitude causes profound hypoxemia during exercise in competitive runners. *Medicine and Science in Sports and Exercise* 27 (Supplement 5), Abstract 623.

Harris, E.K. (1970), Distinguishing physiologic from analytic variation. *Journal of Chronic Disorders* 23, 469.

Harrison, M. (1985), Effects of thermal stress and exercise on blood volume in humans. *Physiology Reviews* 65, 149-209.

Hartley, L.H. Alexander, J.K. Modelski, M. and Grover, R.F. (1967), Subnormal cardiac output at rest and during exercise in residents at 3,100 m altitude. *Journal of Applied Physiology* 23, 839-848.

Hartmann, U. Burrichter, H. Glaser, D. Mader, A. and Oette, K. (1990), Changing in ergometer power outputs and peripheral blood during several high altitude training camps of top class sportsmen and sportswomen. Abstract, XXIV FIMS World Congress of Sports Medicine, Amsterdam.

Haskell, W.L. (1984), The influence of exercise on the concentrations of triglyceride and cholesterol in human plasma. *Exercise and Sport Reviews* 12, 205-244.

Haussinger, D. (1989), Glutamine metabolism in the liver: overview and current concepts. *Metabolism* 38 (Supplement 1), 14-17.

Haymes, E.M. Puhl, J.L. and Temples, T.E. (1986), Training for cross country skiing and iron status. *Medicine and Science in Sports and Exercise* 18, 162-167.

Heimberg, M. Olubavedo, J.O. and Wilcox, H.G. (1985), Plasma lipoprotein and regulation of hepatic metabolism of fatty acids in altered thyroid states. *Endocrinology Reviews* 6, 590-607.

Heistad, D.D. and Abboud, F.M. (1980), Circulatory adjustments to hypoxia. Dickinson W. Richards Lecture. *Circulation* 61, 463-470.

Hietanen, E. (1982), *Regulation of Serum Lipids by Physical Exercise*. In, Hietanen, E. (ed) Boca Raton, Florida: CRC Press, pp 35-54.

Hill, A.V. and Lupton, H. (1923), Muscular exercise, lactic acid and the supply and utilisation of oxygen. *Quarterly Journal of Medicine* 16, 135-171.

Himes, J.H. (1988), Racial variation in physique and body composition. *Canadian Journal of Sports Science* 13, 117-126.

Hochachka, P.W. (1989), The lactate paradox: analysis of underlying mechanisms. *Annals of Sports Medicine* 4, 184-188.

Hochachka, P.W. Stanley, C. Matheson, G.O. McKenzie, D.C. Allen, P.S. and Parkhouse, W.S. (1991), Metabolic and work efficiencies during exercise in Andean natives. *Journal of Applied Physiology* 70, 1720-1730.

Hochachka, P.W. Stanley, C. McKenzie, D.C. Villena, A. and Monge, C. (1992), Enzyme mechanisms for pyruvate-to-lactate flux attenuation: a study of Sherpas, Quechuas and hummingbirds. *International Journal of Sports Medicine* 13 (Supplement 1), S119-S122.

Hogan, M.C. and Welch, H.G. (1986), The effect of altered arterial oxygen tensions on muscle metabolism in dog skeletal muscle during fatiguing work. *American Journal of Physiology* 251, C216-C222.

Hollmann ,W. (1994), The historical development of altitude training and current medical knowledge, in *New studies in athletics, The IAAF Quarterly Magazine for NSA.* Monaco: Multiprint 9 (2), pp 7-13.

Hong, R.W. Rounds, J.D. Helton, W.S. Robinson, M.K. and Wilmore, D.W. (1992), Glutamine preserves liver glutathione after lethal hepatic injury. *Annals of Surgery* 215, 114-119.

Hoppeler, H. Kleinert, E. Schlegel, C. Claassen, H. Howald, H. Kayar, S.R. and Cerretelli, P. (1990), Morphological adaptations of human skeletal muscle to chronic hypoxia. *International Journal of Sports Medicine* 11 (Supplement 1), S3-S9.

Hoppeler, H. (1997), Adaptations to endurance training in the muscle, in Proceedings of, Jeukendrup, A. Brouns, M. and Brouns, F. (eds) *Advances in training and nutrition for endurance sports. From theory to practice.* Papendal, Arnhem, pp 6-7.

Hornbein, T.F. Townes, B.D. Schoene, R.B. Sutton, J.R. and Houston, C.S. (1989), The cost to the central nervous system of climbing to extremely high altitude. *New England Journal of Medicine* 321, 1714-1719.

Hortobágyi, T. Houmard, J.A. Israel, R.G. Carpenter, J.W. Heath, J. and Barakat, H.A. (1993), Effects of exercise cessation on lipids and lipoproteins in distance runners and power athletes. *European Journal of Applied Physiology and Occupational Physiology* 67, 226-230.

Houston, C.S. Sutton, J.R. Cymerman, A. and Reeves, J.T. (1987), Operation Everest II: man at extreme altitude. *Journal of Applied Physiology* 63 (2), 877-882.

Howald, H. Pette, D. Simoneau, J.A. Hoppeler, H. and Cerretelli, P. (1990), Effects of chronic hypoxia on muscle enzyme activities. *International Journal of Sports Medicine* 11 (Supplement 1), S10-S14.

Howell, D.C. (1992) *Statistical Methods for Psychology. 3rd Edition.* Belmont, CA: Duxbury.

Howlett, T.A. and Grossman, A. (1994), The endocrinology of exercise, in, Harries, M. Williams, C. Stanish, W. and Micheli, L. (eds). *Oxford Textbook of Sports Medicine*, London: Oxford University Press, pp 276-282.

Huang, S.Y. Sun, S. Droma, T. Zhuang, J. Tao, J.X. McCullough, R.G. McCullough, R.E. Micco, A.J. Reeves, J.T. and Moore, L.G. (1992), Internal carotid arterial flow velocity during exercise in Tibetan and Han residents of Lhasa (3,658 m). *Journal of Applied Physiology* 73, 2638-2642.

Huebers, H.A. and Finch, C.A. (1987), The physiology of transferrin and transferrin receptors. *Physiological Reviews* 67, 520-582.

Hughson, R.L. and Kowalchuk, J.M. (1995), Kinetics of oxygen uptake for submaximal exercise in hyperoxia, normoxia and hypoxia. *Canadian Journal of Applied Physiology* 20 (2), 198-210.

Hultgren, H.N. (1978), High altitude pulmonary edema in, Staub, N.C. (ed), *Lung, Water and Solute Exchange*. New York: Dekker, pp 437-469.

Ingjer, F. and Myhre, K. (1992), Physiological effects of altitude training on elite male cross-country skiers. *Journal of Sports Sciences* 10, 37-47.

Israel, S. (1976), Zur problematik des Obertrainings aus interinistischer und leistungphysiologischer sicht. *Medicine in Sports* 16, 1-12.

Itoh, H. and Ohkuwa, T. (1990), Peak blood ammonia and lactate after submaximal, maximal and supramaximal exercise in sprinters and long-distance runners. European *Journal of Applied Physiology* 60 (4), 271-276.

Jackson, M.J. Edwards, R.H.T. and Symons, M.C.R. (1985), Electron spin resonance studies of intact mammalian skeletal muscle. *Biochemistry and Biophysics Acta* 847, 185-190.

Jackson, M.J. (1996), Free radicals, exercise and health, in, Maughan, R.J. and Shirreffs, S.M. (eds). *Biochemistry of Exercise IX*. Champaign, Illinois: Human Kinetics, pp 459-464.

Jakeman, P.M. and Davies, B. (1979), The characteristics of a low resistance breathing valve designed for the measurement of high aerobic capacity. *British Journal of Sports Medicine* 13, 81-83.

Jakeman, P.M. Winter, E.M. and Doust, J. (1994), A review of research in sports physiology. *Journal of Sports Sciences* 12, 33-60.

Janssen, G.M.E., van Kranenburg, G. and Geurten, P. (1988), Gender difference in decline of urea concentration during the first 2-3 days post marathon. *Canadian Journal of Sports Sciences* 13, 18P.

Jelkmann, W. (1986), Renal erythropoietin: properties and production. *Reviews of Physiology, Biochemistry and Pharmacology* 104, 139-215.

Jenkins, R. and Mowbray, J. (1991), *Post Viral Fatigue Syndrome*, London: Wiley, pp 32.

Ji, L.L. Lennon, D.L.F. Kochan, R.G. Nagle, F.J. and Lardy, H.A. (1986), Enzymatic adaptation to physical training under β-blockade in the rat: evidence of β_2-adrenergic mechanism in skeletal muscle. *Journal of Clinical Investigations* 78, 771-778.

Joyner, M.J. (1991), Modeling: Optimal marathon performance on the basis of physiological factors. *Journal of Applied Physiology* 70, 683-687.

Kaplowitz, N. Aw, T.Y. and Ookhtens, M. (1985), The regulation of hepatic glutathione. *Annual Reviews in Pharmacology and Toxicology* 25, 715-744.

Kasperek, G.J. and Snider, R.D. (1987), Effect of exercise intensity and starvation on activation of branched-chain keto acid dehydrogenase by exercise. *American Journal of Physiology* 252, E33-E37.

Katz, A. Broberg, S. and Sahlin, K. (1986), Muscle ammonia and amino acid metabolism during dynamic exercise in man. *Clinical Physiology* 6, 365-379.

Katz, A. and Sahlin, K. (1989), Effect of hypoxia on glucose metabolism in human skeletal muscle during exercise. *Acta Physiologica Scandinavica* 136, 377-382.

Kavanagh, M.F. Jacobs, I. Pope, J. Symons, D. and Hermiston, A. (1986), The effect of hypoxia on performance of the wingate anaerobic power test (Wan T). *Canadian Journal of Applied Sports Science* 11, 22P.

Kayser, B. Hoppeler, H. Claassen, C. and Cerretelli, P. (1991), Muscle structure and performance capacity of Himalayan Sherpas. *Journal of Applied Physiology* 70, 1938-1942.

Kayser, B. (1992), Nutrition and high altitude exposure. *International Journal of Sports Medicine* 13 (Supplement 1), S129-S132.

Kayser, B. Ferretti, B. Binzoni, T. and Grassi, B. (1993a), Maximum lactic capacity at high altitude: influence of bicarbonate loading. *Journal of Applied Physiology* 70, 1938-1942.

Kayser, B. Bokenkamp, R. and Binzoni, T. (1993), Alpha-motor neuron excitability at high altitude. *European Journal of Applied Physiology and Occupational Physiology* 66, 1-4.

Kayser, B. Narici, M. Milesi, S. Grassi, B. and Cerretelli, P. (1993c), Body composition and maximum alactic anaerobic performance during a one month stay at high altitude. *International Journal of Sports Medicine* 14 (5), 244-247.

Kayser, B. Narici, M. Binzoni, T. Grassi, B. and Cerretelli, P. (1994), Fatigue and exhaustion in chronic hypobaric hypoxia: influence of exercising muscle mass. *Journal of Applied Physiology* 76, 634-640.

Kayser, B. (1994), Nutrition and energetics of exercise at altitude. Theory and possible practical implications. *Sports Medicine* 17 (5), 309-323.

Kayser, B. Hoppeler, H. Desplanches, D. Marconi, C. Broers, B. and Cerretelli, P. (1996), Muscle ultrastructure and biochemistry of lowland Tibetans. *Journal of Applied Physiology* 81 (1), 419-425.

Keast, D. Cameron, K. and Morton, A.R. (1988), Exercise and the immune response. *Sports Medicine* 5, 248-267.

Keast, D. Arstein, D. and Harper, W. (1995), Depression of plasma glutamine following exercise stress and its possible influence on the immune system. *Medical Journal of Australia* 162, 15-18.

Kinnear, P.R. and Gray, C.D. (1994), *SPSS for Windows*, East Sussex: Psychology Press Publishers.

Kitaev, M.I. and Tokhtabayev, A.G. (1981), T and B lymphocytes as related to adaptation to high altitudes (Russian). *Kosm Biol Aviaksom Med* 15, 87-89.

Klausen, K. Robinson, S. Michael, E.D. and Myhre, L.G. (1966), Effect of high altitude on maximal working capacity. *Journal of Applied Physiology* 21 (4), 1191-1194.

Klausen, T. Mohr, T. Ghisler, U. and Nielsen, O.J. (1991), Maximal oxygen uptake and erythropoietic responses after training at moderate altitude. *European Journal of Applied Physiology and Occupational Physiology* 62, 376-379.

Klein, S. Coyle, E.F. and Wolfe, R.R. (1994), Fat metabolism during low-intensity exercise in endurance-trained and untrained men. *American Journal of Physiology* 267 (Endocrinology and Metabolism 30), E934-E940.

Klokker, M. Kharazmi, A. Galbo, H. Bygbjerg, I. and Pedersen, B.K. (1993), Influence of in vivo hypobaric hypoxia on function of lymphocytes, neutrocytes, natural killer cells, and cytokines. *Journal of Applied Physiology* 74 (3), 1100-1106.

Knaupp, W. Khilani, S. Sherwood, J. Scharf, S. and Steinberg, H. (1992), Erythropoietin response to acute normobaric hypoxia in humans. *Journal of Applied Physiology* 73 (3), 837-840.

Kobayashi, Y. Ando, Y. Takeuchi, S. Takemura, K. and Okuda, N. (1980), Effects of heat acclimation of distance runners in a moderately hot environment. *European Journal of Applied Physiology and Occupational Physiology* 45, 189-198.

Koistinen, P. Takala, T. Martikkala, V. and Leppalouto, J. (1995), Aerobic fitness influences the response of maximal oxygen uptake and lactate threshold in acute hypobaric hypoxia. *International Journal of Sports Medicine* 26 (2), 78-81.

Kokkinos, P.F. Holland, J.C. Narayan, P. Colleran, J.A. Dotson, C.O. and Papademetriou, V. (1995), Miles run per week and high-density lipoprotein cholesterol levels in healthy, middle-aged men. *Archives of International Medicine* 155, 415-420.

Kollias, J. Buskirk, E.R. Akers, R.F. Prokop, E.K. Baker, P.T. and Picon-Reategui, E. (1968), Work capacity of long-time residents and newcomers to altitude. *Journal of Applied Physiology* 24 (6), 792-799.

Kouskoulu, M. Coutts, K.D. Mekjavic, I.B. Cooper, T.K. Jespersen, D.K. Hopkins, S.R. and McKenzie, D.C. (1992), Arterial hypoxemia and performance during intense exercise. *Medicine and Science in Sports and Exercise* 24 (Supplement 5), Abstract 617.

Krantz, S.B. (1991), Erythropoietin. *Blood* 77, 419-434.

Krebs, H.A. (1980), Glutamine metabolism in the animal body, in, Mora, J. and Palacios, R. (eds). *Glutamine: metabolism, enzymology and regulation*. New York: Academic Press, 319-329.

Krogh, A. (1919), The rate of diffusion of gases through animal tissues with some remarks about the coefficient of invasion. *Journal of Physiology London* 52, 391-422.

Kuipers, H. and Keizer, H.A. (1988), Overtraining in elite athletes. *Sports Medicine* 6, 79-92.

Kyle, J.M. (1994), Exercise-induced pulmonary syndromes. *Medical Clinics of North America* 78 (2), 413-421.

Lahiri, S. Maret, K.H. Sherpa, M.G. and Peters, R.M. (1984), Sleep and periodic breathing at high altitude: Sherpa natives versus sojourners, in, West, J.B. and Lahiri, S. (eds) *High altitude and man*, American Physiological Society, Bethesda, pp 73-90.

Laitinen, H. Alopaeus, K. Heikkinen, R. Hietanen, H. Mikkelson, L. Tikkanen, H. and Rusko, H.K. (1994), Acclimatization to living in normobaric hypoxia and training in normoxia at sea level in runners. *Medicine and Science in Sports and Exercise* 27 (5), Abstract 617.

Lakka, T.A. and Salonen, J.T. (1992), Physical activity and serum lipids: a cross-sectional population study in eastern Finnish men. *American Journal of Epidemiology* 136, 806-818.

Lakomy, H.K.A. (1994), Assessment of anaerobic power, in, Harries, M.; Williams, C. Stanish, W. and Micheli, L. (eds). *Oxford Textbook of Sports Medicine*, London: Oxford University Press, pp 180-187.

Lawler, J. Powers, S.K. and Thompson, D. (1988), Linear relationship between VO_2 Max and VO_2 Max decrement during exposure to acute hypoxia. *Journal of Applied Physiology* 64 (4), 146-149.

Lawrence, D.L. and Shenker, Y. (1991), Effect of hypoxic exercise on atrial natriuretic factor and aldosterone regulation. *American Journal of Hypertension* 4, 341-347.

Lehmann, M. Baumgartl, P. Wiesenack, C. Seidel, A. Baumann, H. Fischer, S. Spöri, U. Gendrisch, G. Kaminski, R. and Keul, J. (1992), Training-overtraining: influence of a defined increase in training volume vs training intensity on performance, catecholamines and some metabolic parameters in experienced middle- and long-distance runners. *European Journal of Applied Physiology and Occupational Physiology* 64, 169-177.

Lemanske, R.F. (1989), Exercise-induced asthma in children. *Sports Science Exchange* 2 (17).

Lemon, P.W.R. and Mullin, J.P. (1980), Effect of initial muscle glycogen levels on protein catabolism during exercise. *Journal of Applied Physiology* 48 (4), 624-629.

Lemon, P.W.R. Deutsch, D.T. and Payne, W.R. (1989), Urea production during prolonged swimming. *Journal of Sports Sciences* 7, 241-246.

Lemon, P.W.R. (1991), Effect of exercise on protein requirements. *Journal of Sports Sciences* 9, 53-70.

Lenfant, C. and Sullivan, K. (1971), Adaptation to high altitude. *New England Journal of Medicine* 284 (23), 1298-1309.

Levine, B.D. Engfred, K. Friedman, D.B. Kjaer, M. Saltin, B. Clifford, P.S. and Secher, N.H. (1990), High altitude endurance training: Effect on aerobic capacity and work performance. *Medicine and Science in Sports and Exercise* 22 (2), Abstract 209.

Levine, B.D. Stray-Gundersen, J. Duhaime, G. Snell, P.G. and Friedman, D.B. (1991), Living high - training low: The effect of altitude acclimatization / normoxic training in trained runners. *Medicine in Science in Sports and Exercise* 23 (4), Abstract 145.

Levine, B.D. and Stray-Gundersen, J. (1992), A practical approach to altitude training: Where to live and train for optimal performance enhancement. *International Journal of Sports Medicine* 13, S209-212.

Levine, B.D. Roach, R.C. and Houston CS. (1992), Work and training at altitude, in Houston, et al, (eds). *Hypoxia and mountain medicine*, Burlington: Queen City Printers, pp 192-201.

Levine, B.D. Friedman, D.B. Engfred, K. Hanel, B. Kjaer, M. Clifford, P.S. and Secher, N.H. (1992), The effect of normoxic or hypobaric hypoxic endurance training on the hypoxic ventilatory response. *Medicine and Science in Sports and Exercise* 24 (7), 769-775.

Levine, B.D. Friedmann, B. and Stray-Gundersen, J. (1996), Confirmation of the "High-low" hypothesis: Living at altitude-Training near sea level improves sea level performance. *Medicine and Science in Sports and Exercise* 28 (5), Abstract 742.

Levine, B.D. and Stray-Gundersen, J. (1997), "Living high-training low": effect of moderate-altitude acclimatization with low-altitude training on performance. *Journal of Applied Physiology* 83 (1), 102-112.

Levitzky, M.G. (1995), *Pulmonary physiology, fourth edition.* USA: Mc Graw-Hill, pp 74.

Linde, F. (1987), Running and upper respiratory tract infections. *Scandinavian Journal of Sports Science* 9, 21-23.

Loat, C.E.R. and Rhodes, E.C. (1993), Relationship between the lactate and ventilatory thresholds during prolonged exercise. *Sports Medicine* 15 (2), 104-115.

Loeppky, J.A. and Bynum, W.A. (1970), Effects of periodic exposure to hypobaria and exercise on physical work capacity. *Journal of Sports Medicine and Physical Fitness* 10, 238-247.

Loeppky, J.A. Roach, R.C. Selland, M.A. Scotto, P. Greene, E.R. and Luft, U.C. (1993), Effects of prolonged head-down bed rest on physiological responses to moderate hypoxia. *Aviation, Space and Environmental Medicine* 64, 275-286.

Lohman, T.G. (1981), Skinfolds and body density and their relation to body fatness: a review. *Human Biology* 53, 181-225.

Lokey, E.A. and Tran, Z.V. (1989), Effects of exercise training on serum lipid and lipoprotein concentrations in women: a meta-analysis. *International Journal of Sports Medicine* 10 (6), 424-429.

Lund, P. (1974), L-Glutamine. Determination with glutaminase and glutamate dehydrogenase, in, Bergmeyer, H.U. (ed). *Methods of Enzymatic Analysis* 4, New York: Academic Press, pp 1719-1722.

MacDougall, J.D. Green, H.J. Sutton, J.R. Coates, G. Cymerman, A. Young, P.M. and Houston, C.S. (1991), Operation Everest II: structural adaptations in skeletal muscle in response to extreme simulated hypoxia. *Acta Physiologica Scandinavica* 142, 421-427.

Magnusson, B. Hallberg, L. and Rossander, L. (1984), Iron metabolism and sports anaemia. *Acta Medica Scandinavica* 216, 157-164.

Mairbaurl, H. Hasibeder, W. Schobersberger, W. and Raas, E. (1987), Ausbelastungsergometrie in mittleren Hohenlagen und Einflusse verminderter Hb-O_2 Affinitat, in, Bachl, Baumgarl P, Huber G and Keul J (eds). *Die trainings physiologische und klinische Bedeutung der anaeroben Kapazitat. Wien*: Hollinek, pp 365-373.

Mairbaurl, H. Schobersberger, W. Oelz, O. Bartsch, P. Eckardt, K.U. and Bauer, C (1990), Unchanged in-vivo P_{50} at high altitude despite decreased red cell age and elevated 2,3-DPG. *Journal of Applied Physiology* 68 (3), 1186-1194.

Mairbaurl, H. (1994), Red blood cell function in hypoxia at altitude and exercise. *International Journal of Sports Medicine* 15 (2), 51-63.

Mann, H.B. and Whitney, D.R. (1947), On a test whether one of two random variables is stochastically larger than the other. *Annals of Mathematical Statistics* 18, 50-60.

Marieb, E.N. (1989), *Human Anatomy and Physiology*, in Spatz, C.J. Burner, P.S. and Fox, R. (eds). California: Benjamin/Cummings Publishing Company, Inc, pp 574.

Martin, D. and O'Kroy, J. (1993), Effects of acute hypoxia on the VO_2max of trained and untrained subjects. *Journal of Sports Sciences* 11, 37-42.

Martino, M. Myers, K. and Bishop, P. (1995), Effects of 21 days training at altitude on sea-level anaerobic performance in competitive swimmers. *Medicine and Science in Sports and Exercise* 27 (5), Abstract 37.

Masuda, A. Kobayashi, T. and Honda, Y. (1992), Effect of high altitude on respiratory chemosensitivity. *Japanese Journal of Mountaineering Medicine* 12, 177-181.

Matheson, G.O. Allen, P.S. Ellinger, D.C. Hanstock, C.C. Gheorghiu, D. McKenzie, D.C. Stanley, C. Parkhouse, W.S. and Hochachka, P.W. (1991), Skeletal muscle metabolism and work capacity: a ^{31}P-NMR study of Andean natives and lowlanders. *Journal of Applied Physiology* 70, 1963-1976.

Mattila, V. and Rusko, H. (1994), Effect of living high and training low on sea level performance in cyclists. *Medicine and Science in Sports and Exercise* 26 (5), Abstract 928.

Maughan, R.J. (1994), Fluid and electrolyte loss and replacement in exercise, in, Harries, M. Williams, C. Stanish, W. and Micheli, L. (eds). *Oxford Textbook of Sports Medicine*, London: Oxford University Press, pp 82-93.

Maughan, R.J. and Gleeson, M. (1988), Influence of a 36h fast followed by refeeding with glucose, glycerol or placebo on metabolism and performance during prolonged exercise in man. *European Journal of Applied Physiology and Occupational Physiology* 57, 570-576.

Maughan, R. and Shirreffs, S.M. (1997), Preparing athletes for competition in the heat: developing an effective acclimatization strategy. *Gatorade Sports Science Institute* 10 (2).

Mazzeo, R.S. Bender, P.R. and Brooks, G.A. (1989) Dissociation of the catecholamine response during exercise with acute and chronic altitude exposure. *Journal of Applied Physiology* In press.

McLellan, T.M. Kavanagh, M.F. and Jacobs, I. (1990), The effect of hypoxia on performance during 30 s or 45 s of supramaximal exercise. *European Journal of Applied Physiology and Occupational Physiology* 60, 155-161.

McLellan, T.M. and Cheung, K.S.Y. (1992), A comparative evaluation of the anaerobic threshold and the critical power. *Medicine and Science in Sport and Exercise* 24, 543-550.

Meehan, R.T. (1987), Immune suppression at high altitude. *Annals of Emergency Medicine* 16, 974-979.

Meehan, R.T. Duncan, U. Neale, L.S. Taylor, G. Muchmore, H. Scott, N., Ramsey, K. Smith, E. Rock, P. Goldblum, R. and Houston, C. (1988), Operation Everest II: alterations in the immune system at high altitudes. *Journal of Clinical Immunology* 8, 397-406.

Metra, M. Cannella, G. La Canna, G. (1991), Improvement in exercise capacity after correction of anemia in patients with end stage renal failure. *American Journal of Cardiology* 68, 1060-1066.

Meyer, R.A. and Terjung, R.L. (1979), Difference in ammonia and adenylate metabolism in contracting fast and slow muscle. *American Journal of Physiology* 237, C111-C118.

Milledge, J.S. and Lahiri, S. (1967), Respiratory control in lowlanders and Sherpa highlanders at altitude. *Respiratory Physiology* 2, 310-322.

Milledge, J.S. and Lahiri, S. (1967), Respiratory control in lowlanders and Sherpa highlanders at altitude. *Respiratory Physiology* 2, 310-322.

Milledge, J.S. Halliday, D. Pope, C. Ward, P. Ward, M.P. and Williams, E.S. (1976), The effect of hypoxia on muscle glycogen resynthesis in man. *Quarterly Journal of Experimental Physiology* 62, 237-245.

Milledge J.S. and Coates, P.M. (1985), Serum erythropoietin in humans at high altitude and its relation to plasma renin. *Journal of Applied Physiology* 59, 360-364.

Milledge, J.S. (1992b), Salt and water control at altitude. *International Journal of Sports Medicine* 13 (S61-S63).

Milledge, J.S. (1994), High altitude, in, Harries, M. Williams, C. Stanish, W. and Micheli, L. (eds). *Oxford Textbook of Sports Medicine*, London: Oxford University Press, pp 217-230.

Mills, W.J. (1973), Frostbite and hypothermia. Current concepts. *Alaska Medicine* 15, 26-59.

Mizuno, M. Juel, C. Bro-Rasmussen, T. Mygind, E. Schibye, B. Rasmussen, B. and Saltin, B. (1990), Limb skeletal muscle adaptation in athletes after training at altitude. *Journal of Applied Physiology* 68 (2), 496-502.

Morgan, B.J. Alexander, J.K. Nicoli, S.A. and Brammell, H.L. (1990), The patient with coronary heart disease at altitude: observations during acute exposure to 3100 meters. *Journal of Wilderness Medicine* 1, 147-153.

Morgan, D.W. and Craib, M. (1992), Physiological aspects of running economy. *Medicine and Science in Sports and Exercise* 24 (2), 456-461.

Mortimer, E.A. Monson, R.R. and MacMahon, B. (1977), Reduction of mortality from coronary heart disease in men residing at high altitude. *New England Journal of Medicine* 296, 581-585.

Nachtigall, D. Nielsen, P. Fischer, R. Engelhardt, R. and Gabbe, E.E. (1996), Iron deficiency in distance runners. A reinvestigation using ^{59}Fe-labelling and non-invasive liver iron quantification. *International Journal of Sports Medicine* 17, 473-479.

Nagawa, T. Kita, H. Aoki, J. Maeshima, T. and Shiozawa, K. (1968), *Asian Medicine* 11, 619-633.

Narici, M.V. and Kayser, B. (1995), Hypertrophic response of human skeletal muscle to strength training in hypoxia and normoxia. *European Journal of Applied Physiology and Occupational Physiology* 70, 213-219.

Nestel, P.J. Podkolinski, M. and Fidge, N.H. (1979), Marked increase in high density lipoproteins in mountaineers. *Atherosclerosis* 34, 193-196.

Neureither, M. Bartsch, P. and Strobel, G. (1996), Effect of acute mild hypoxia during submaximal exercise on plasma free and sulfoconjugated catecholamines. *International Journal of Sports Medicine* 17 (S26), Abstract.

Newsholme, E.A. and Leech, A.R. (1983) *Biochemistry for the Medical Sciences*, in Wiley, J. (Editor), Chichester, UK.

Newsholme, E.A. Crabtree, B. and Ardawi, M.S.M. (1985), Glutamine metabolism in lymphocytes: its biochemical, physiological and clinical importance. *Quarterly Journal of Experimental Physiology* 70, 473-489.

Newsholme, E.A. Crabtree, B. and Ardawi, M.S.M. (1985), The role of high rates of glycolysis and glutamine utilization in rapidly-dividing cells. *Bioscience Reports* 4, 393-400.

Newsholme, E.A. Acworth, I.N. and Blomstrand, E. (1987), Amino acids, brain neurotransmitters and a functional link between muscle and brain that is important in sustained exercise, in, Benzi, G. (ed). *Advances in Myochemistry*, London: John Libbey Eurotext, pp 127-133.

Newsholme, E.A. Newsholme, P. Curi, R. Challoner, E. and Ardawi, M.S.M. (1988), A role for muscle in the immune system and its importance in surgery, trauma, sepsis and burns. *Nutrition* 4 (4), 261-268.

Newsholme, E.A. and Castell, L.M. (1996), Can amino acids influence exercise performance in athletes? In, Steinacker and Ward (eds). *The Physiology and Pathophysiology of Exercise Tolerance*, New York: Plenum Press, pp 269-274.

Newsholme, P. Gordon, S. and Newsholme, E.A. (1987), Rates of utilization and fates of glucose, glutamine, pyruvate, fatty acids and ketone bodies by mouse macrophages. *Biochemistry Journal* 242, 631.

Nielsen, O.J. (1988), Determination of human erythropoietin by radioimmunoassay. Method and clinical data. *Clinical Chemistry Acta* 176, 303-314.

Nieman, D.C. Butler, J.V. Pollett, L.M. Dietrich, S.J. and Lutz, R.D. (1989), Nutrient intake of marathon runners. *Journal of the American Dietetic Association* 89, 1273-1278.

Nieman, D.C. Johanssen, L.M. Lee, J.W. and Arabatzis, K. (1990), Infectious episodes in runners before and after the Los Angeles Marathon. *Journal of Sports Medicine and Physical Fitness* 30, 316-328.

Nieman, D.C.(1996), Effect of long-term training on the immune system and on resistance to infectious diseases, in, Maughan, R.J. and Shirreffs, S.M. (eds). *Biochemistry of Exercise IX*. Champaign, Illimois: Human Kinetics, pp 383-398.

Niermeyer, S. Yang, P. Drolkar, S. Zhuang, J. and Moore, L.G. (1995), Arterial oxygen saturation in Tibetan and Han infants born in Lhasa, Tibet. *New England Journal of Medicine* 333, 1248-1252.

Noakes, T. (1988), Implications of exercise testing for prediction of athletic performance: a contemporary perspective. *Medicine and Science in Sports and Exercise* 20 (4), 319-330.

Noakes, T.D. (1986), *The Lore of Running*. Cape Town: Oxford University Press.

Nummela, A. Jouste, P. and Rusko, H. (1996), Effect of living high and training low on sea level anaerobic performance in runners. *Medicine and Science in Sports and Exercise* 28. Abstract 740.

Otis, A.B. (1954) The work of breathing. *Physiological Reviews* 34, 449-458.

O'Toole, M.L. Hiller, W.D.B. and Douglas, P.S. (1989), Serum ferritin as a measure of triathlon stress. Proceedings from First IOC World Congress on Sports Sciences, Colorado Springs, pp 85-86.

Parry-Billings, M. Blomstrand, E. McAndrew, N. and Newsholme, E. (1990), A communicational link between skeletal muscle, brain and cells of the immune system. *International Journal of Sports Medicine* 11 (S122-S128).

Parry-Billings, M. Evans, J. Calder, P. and Newsholme, E.A. (1990), Does glutamine contribute to immunosupression after major burns? Lancet 336, 523-525.

Parry-Billings, M. Budgett, R. Koutedakis, Y. Blomstrand, E. and Brooks, S. (1992), Plasma amino acid concentrations in the overtraining syndrome: possible effects on the immune system. *Medicine and Science in Sports and Exercise* 24, 1353-1358.

Pate, R.R. (1988) Special consideration for exercise in cold weather. *Sports Science Exchange* 10 (2).

Pate, R.R. Macera, C.A. Bailey, S.P. Bartoli, W.P. and Power, K.E. (1992), Physiological, anthropometric and training correlates of running economy. *Medicine and Science in Sports and Exercise* 24, 1128-1123.

Pedersen, B.K. (1996), Acute, time-limited exercise stress and the immune system: role of stress hormones, in, Maughan, R.J. and Shirreffs, S.M. (eds). *Biochemistry of Exercise IX.* Champaign, Illinois: Human Kinetics, pp 497-510.

Penaloza, D. (1971), Discussion, in, Porter, R. and Knight, J. (eds). *High Altitude Physiology: Cardiac and Respiratory Aspects*, Edinburgh: Churchill Livingstone, pp 169.

Peronnet, F. Thibault, G. and Cousineau, D.L. (1991), A theoretical analysis of the effect of altitude on running performance. *Journal of Applied Physiology* 70, 399-404.

Peters, E.M. (1990), Altitude fails to increase susceptibility of ultramarathon runners to post-race upper respiratory tract infections. *South African Journal of Sports Medicine* 5, 4-8.

Peters, E.M. Goetzsche, J.M. Grobbelaar, B. and Noakes, T.D. (1993), Vitamin C supplementation reduces the incidence of postrace symptoms of upper-respiratory-tract infection in ultramarathon runners. *American Journal of Clinical Nutrition* 57, 170-174.

Piehl, K. Adolfsson, S. and Nazar, K. (1974), Glycogen storage and glycogen synthetase activity in trained and untrained muscle of man. *Acta Physiologica Scandinavica* 90, 779-788.

Pitts, R.F. Pilkington, L.A. and MacLeod, M.B. (1972), Metabolism of glutamine by the intact functioning kidney of the dog. *Journal of Clinical Investigation* 51, 557-565.

Pollock, N.W. Davies, B. and Godfrey, R.J. (1996), Urine osmolality in world class athletes making weight. *The Physiologist* 39 (5), Abstract 53.

Preedy, V.R. Smith, D.M. and Sugden, P.H. (1985), The effects of 6 hours hypoxia on protein synthesis in rat tissues in vivo and in vitro. *Journal of Biochemistry* 228, 179-185.

Préfaut, C. Anselme-Poujol, F. and Caillaud, C. (1997), Inhibition of histamine release by nedocromil sodium reduces exercise-induced hypoxemia in master athletes. *Medicine and Science in Sports and Exercise* 29 (1), 10-16.

Pronk, N.P. (1993), Short term effects of exercise on plasma lipids and lipoproteins in humans. *Sports Medicine* 16 (6), 431-448.

Pugh, L.G.C.E. (1957), Resting ventilation and alveolar air on Mount Everest: with remarks on the relation of barometric pressure to altitude in mountains. *Journal of Physiology, London* 135, 590-610.

Pugh, L.G.C.E. (1964), Cardiac output in muscular exercise at 5,800 m (19,000 ft). *Journal of Applied Physiology* 19 (3), 441-447.

Pugh, L.G.C.E. Gill, M.B. Lahiri, S. Milledge, J.S. Ward, M.P. and West, J.B. (1964), Muscular exercise at great altitudes. *Journal of Applied Physiology* 19 (3), 431-440.

Pugh, L.G.C.E. (1965), Altitude and athletic performance. *Nature* 207, 1397-1398.

Puranen, A.S. and Rusko, H.K. (1995), On-and-off responses of EPO, reticulocytes, 2,3-DPG and plasma volume to living high, training low. *Medicine and Science in Sports and Exercise* 28 (5), Abstract 947.

Quick, J. Eichenberger, A. and Binswanger, U. (1992), Stimulation of erythropoietin in renal insufficiency by hypobaric hypoxia. *Nephrology, Dialysis and Transplantation* 7 (10), 1002-1006.

Radak, Z. Lee, K. Choi, W. Sunoo, S. Kizaki, T. Oh-ishi, S. Suzuki, K. Taniguchi, N. Ohno, H. and Asano, K. (1994), Oxidative stress induced by intermittent exposure at a simulated altitude of 4000 m decreases mitochondrial superoxide dismutase content in soleus muscle of rats. *European Journal of Applied Physiology and Occupational Physiology* 69 (5), 392-395.

Rahkila, P. and Rusko, H. (1982), Effects of high altitude training on muscle enzyme activities and physical performance characteristics of cross-country skiers, in Komi, P. (ed). *Exercise and Sport Biology*. Champaign, Illinois: Human Kinetics, pp 143-151.

Rahn, H. and Otis, A.B. (1949), Man's respiratory response during and after acclimatization to high altitude. *American Journal of Physiology* 147, 444-462.

Ramos, D.A. Kruger, H. Muro, M. and Arias-Stella, J. (1967), Patalogica del hombre nativo de las grande alturas: investigacion de las causes de muetre en 300 autopsias. *Bon. Sanit. Panam* 62, 497-507.

Ratzin Jackson, C.G. and Sharkey, B.J. (1988), Altitude, training and human performance. *Sports Medicine* 6, 279-284.

Raven, P.B. and Hagan, R.D. (1994), Cardiovascular responses to exercise and training, in, Harries, M. Williams, C. Stanish, W. and Micheli, L. (eds). *Oxford Textbook of Sports Medicine*. London: Oxford University Press, pp 161-172.

Raynaud, J. Martineaud, J.P. Bordachar, J. Tillous, M.C. and Durand, J. (1974), Oxygen deficit and debt in submaximal exercise at sea level and high altitude. *Journal of Applied Physiology* 37, 43-48.

Reeves, J.T. Grover, R.F. and Cohn, J.E. (1967), Regulation of ventilation during exercise at 10, 200 ft in athletes born at low altitude. *Journal of Applied Physiology* 22 (3), 546-554.

Reeves, J.T. Groves, B.M. Sutton, J.R. Wagner, P.D. Cymerman, A. Malconian, M.K. Rock, P.B. Young, P.M. and Houston, C.S. (1987), Operation Everest II: preservation of cardiac function at extreme altitude. *Journal of Applied Physiology* 63 (2), 531-539.

Reeves, J.T. Wolfel, E.E. Green, H.J. Mazzeo, R.S. Young, A.J. Sutton, J.R. and Brooks, G.A. (1992), Oxygen transport during exercise at altitude and the lactate paradox: Lessons from Operation Everest II and Pikes Peak. *Exercise and Sport Science Reviews* 20, 275-296.

Reilly, T. and Brooks, G.A. (1982), Investigation of circadian rhythms in metabolic responses to exercise. *Ergonomics* 25, 1093-1197.

Reilly, T. and Brooks, G.A. (1986), Exercise and the circadian variation in body temperature measures. *International Journal of Sports Medicine* 7, 358-362.

Reilly, T. (1994), Circadian rhythms, in, Harries, M. Williams, C. Stanish, W. and Micheli, L. (eds). *Oxford Textbook of Sports Medicine*, London: Oxford University Press, pp 238-254.

Rejnafarje, C. Lozano, R. and Valdivieso, J. (1959), The polycythemia of high altitudes: iron metabolism and related aspects. *Blood* 14, 433-455.

Reynafarje, B. (1961), The influence of altitude changes on intestinal iron absorption. *Journal of Laboratory and Clinical Medicine* 57, 848.

Reynafarje, B. (1962), Myoglobin content and enzymatic activity of muscle and altitude adaptation. *Journal of Applied Physiology* 17, 301-305.

Rennie, M.J. Edwards, R.H.T. and Krywawych, S. (1981), Effect of exercise on protein turnover in man. *Clinical Science* 61, 627-639.

Rennie, M.J. Babij, P. Sutton, J.R. Tonkins, W.J. Read, W.W. Ford, C. and Halliday, D. (1983), Effects of acute hypoxia on forearm leucine metabolism, in, Sutton, J.R. (ed). *Hypoxia, exercise and altitude*. New York: LISS, pp 317-323.

Resina, A. Gatteschi, L. Giamberardino, M.A. Rubenni, M.G. Trabassi, E. and Troni, M.G. (1988), Comparison of RBC indices and serum iron parameters in trained runners and control subjects. *Haematologica* 73, 449-454.

Rhoades, R.A. Packer, C.S. Roepke, D.A. Jin, N. and Meiss, R.A. (1990), Reactive oxygen species alter contractile properties of pulmonary arterial smooth muscle. *Canadian Journal of Physiology and Pharmacology* 68, 1581-1589.

Richalet, J.P. Keromes, A. and Dersch, B. (1988), Physiological characteristics of mountaineers at high altitude. *Science in Sports* 3, 89-108.

Richalet, J.P. Larmignat, P. Rathat, C. Kéromès, A. Baud, P. and Lhoste, F. (1988), Decreased cardiac response to isoproterenol infusion in acute and chronic hypoxia. *Journal of Applied Physiology* 65, 1957-1961.

Richalet, J.P. Rutgers, V. and Bouchet, P. (1989), Diurnal variation of acute mountain sickness, color vision and plasma cortisol and ACTH at high altitude. *Aviation, Space and Environmental Medicine* 60, 105-111.

Richalet, J.P. Marchal, M. Lamberto, C. Le Trong, J.L. Antezana, A.M. and Cauchy, E. (1992), Alteration of aerobic and anaerobic performance after 3 weeks at 6542m (Mt. Sajama). *International Journal of Sports Medicine* 13, Abstract.

Richalet, J.P. Souberbielle, J.C. and Antezana, A.M. (1993), Control of erythropoiesis in humans during prolonged exposure to the altitude of 6542 m. *American Journal of Physiology* 266, R756-R764.

Roberts, D. and Smith, D.J. (1992), Serum erythropoietin of elite swimmers training at moderate altitude, in, Maclaren, D. Reilly, T. and Lees, A. (eds). *Biomechanics and medicine in swimming. Swimming Science VI.* London: Chapman and Hall Medical, pp 341-345.

Roberts, A.C. Brooks, G.A. Butterfield, G.E. Wolfel, E.E. and Reeves, J.T. (1994), Acclimatization to 4,300 m altitude decreases reliance on fat as a substrate and increases dependency on blood glucose. *Medicine and Science in Sports and Exercise* 26 (5), Abstract 121.

Robertson, R.J. Gilcher, R. Metz, K.F. Skrinar, G.S. Allison, T.G. Bahnson, H.T. Abbott R.A. Becker, R. and Falkel, J.E. (1982), Effect of induced erythrocythemia on hypoxia tolerance during physical exercise. *Journal of Applied Physiology* 53 (2), 490-495.

Robertson, R.J. Gilcher, R. Metz, K.F. Caspersen, C.J. Allison, T.G. Abbott, R.A. Skrinar, G.S. Krause, J.R. and Nixon, P.A. (1984), Hemoglobin concentration and aerobic work capacity in women following induced erythrocythemia. *Journal of Applied Physiology*. 57 (2), 568-575.

Robertson, R.J. Gilcher, R. Metz, K.F. Caspersen, C.J. Allison, T.G. Abbott, R.A. Skrinar, G.S. Krause, J.R. and Nixon, P.A. (1988), Effect of simulated altitude erythrocythemia in women on haemoglobin flow rate during exercise. *Journal of Applied Physiology* 64, 1644-1649.

Robinson, S. (1938) Experimental studies of physical fitness in relation to age. *Arbeitsphysiologie* 10, 251.

Rose, M.S. Houston, C.S. and Fulco, C.S. (1988), Operation Everest II: nutrition and body composition. *Journal of Applied Physiology* 65, 2545-2551.

Roskamm, H. Landry, F. Samek, L. Schlager, M. Weidemann, H. and Reindell, H. (1969), Effects of a standardized ergometer training program at three different altitudes. *Journal of Applied Physiology* 27 (6), 840-847.

Rosser, B.W.C. and Hochachka, P.W. (1993), Metabolic capacity of muscle fibers from high-altitude natives. *European Journal of Applied Physiology and Occupational Physiology* 67, 513-517.

Roth, D.A. and Brooks, G.A. (1990), Lactate transport is mediated by a membrane-bound carrier in rat skeletal muscle sarcolemmal vesicles. *Archives of Biochemistry and Biophysics* 279, 377-385.

Roth, D.A. (1991), The sarcolemmal lactate transporter: transmembrane determinants of lactate flux. *Medicine and Science in Sports and Exercise* 23 (8), 925-934.

Rowbottom, D.G. Keast, D. and Goodman, C. (1995), The haematological, biochemical and immunological profile of athletes suffering from the Overtraining Syndrome. *European Journal of Applied Physiology and Occupational Physiology* 70, 502-509.

Rowbottom, D.G. Keast, D. and Morton, A. (1996), The emerging role of glutamine as an indicator of exercise stress and overtraining. *Sports Medicine* 21 (2), 80-97.

Rowell, L.B. Taylor, H.L. and Wang, Y. (1964), Saturation of arterial blood with oxygen during maximal exercise. *Journal of Applied Physiology* 19, 284-286.

Rowell, L.B. *Human circulation: regulation during physical stress*. New York: Oxford University Press, pp 1-416.

Rusko, H.K. Kirvesniemi, H. Paavolainen, L. Vahasoyrinki, P. and Kyro, K.P. (1996), Effect of altitude training on sea level aerobic and anaerobic power of elite athletes. *Medicine and Science in Sports and Exercise* 28 (5), Abstract 739.

Ryan, A.J. Brown, R.L. Frederick, E.C. Falsetti, H.L. and Burke, E. (1983), Overtraining of athletes. *The Physician and Sports Medicine* 11, 93-110.

Sahlin, K. Katz, A. and Broberg, S. (1990), Tricarboxylic acid cycle intermediates in human muscle during prolonged exercise. *American Journal of Physiology* 259, C834-841.

Sahlin, K. (1994), Acid-base balance during high intensity exercise. In, Harries, M. Williams, C. Stanish, W. and Micheli, L. (eds). *Oxford Textbook of Sports Medicine*, London: Oxford University Press, pp 46-52.

Sahlin K. (1996), Ammonia metabolism in humans during exercise, in, Maughan, R.J. and Shirreffs, S.M. (eds). *Biochemistry of Exercise IX*. Champaign, Illinois: Human Kinetics, pp 497-510.

Salo, D.C. Donovan, C.M. and Davies, K.J.A. (1991), HSP70 and other possible heat shock or oxidative stress proteins are induced in skeletal muscle, heart and liver during exercise. *Free Radicals in Biology and Medicine* 11, 239-246.

Saltin, B. (1967), Aerobic and anaerobic work capacity at 2,300 metres. *Medical Thoracics* 24, 205-210.

Saltin, B. Grover, R.F. Blomqvist, C.G. Hartley, L.H. and Johnson, R.L. (1968), Maximal oxygen uptake and cardiac output after 2 weeks at 4,300 m. *Journal of Applied Physiology* 25 (3), 400-409.

Saltin, B. and Gollnick, P.D. (1983), Skeletal muscle adaptability: significance for metabolism and performance, in Peachey, L.D. and Adrian, R.H. (eds). *Handbook of physiology-skeletal muscle. American Physiological Society*, Baltimore: Williams and Wilkins, pp 555-631.

Saltin, B. (1988), Limitations to performance at altitude, in, Sutton, J.R. Houston, C.S. and Coates, G. (eds). *Hypoxia: The Tolerable Limits*, Indianapolis: Benchmark, pp 9-34.

Saltin, B. and Costill, D.L. (1988), Fluid and electrolyte balance during prolonged exercise, in, Horton, E.S. and Terjung, R.L. (eds). *Exercise, nutrition and metabolism*. New York: Macmillan, pp 150-158.

Saltin, B., Kim C.K. Terrados, N. Larsen, H. Svedenhag, J. and Rolf, C.J. (1995), Morphology, enzyme activities and buffer capacity in leg muscles of Kenyan and Scandinavian runners. *Scandinavian Journal of Medicine and Science in Sports* 5, 222-230.

Saltin, B. Larsen, H. Terrados, N. Bangsbo, J. Bak, T. Kim, C.K. Svedenhag, J. and Rolf, C.J. (1995), Aerobic exercise capacity at sea level and at altitude in Kenyan boys, junior and senior runners compared with Scandinavian runners. *Scandinavian Journal of Medicine and Science in Sports* 5, 209-221.

Saltin, B. (1996), Exercise and the environment: Focus on altitude. *Research Quarterly for Exercise and Sport* 67, 1-10.

Salway, J.G. (1995) *Metabolism at a glance*. London: Blackwell Scientific Publications, pp 40-41.

Samaja, M. Brenna, L. Allibardi, S. and Cerretelli, P. (1993), Human red blood cell aging at 5,050-m altitude: a role during adaptation to hypoxia. *Journal of Applied Physiology* 75 (4), 1696-1701.

Sanchez, C. Merino, C. and Figallo, M. (1970), Simultaneous measurement of plasma volume and cell mass in polycythemia of high altitude. *Journal of Applied Physiology* 28, 775-778.

Savard, G.K. Areskog, N.H. and Saltin, B. (1995), Cardiovascular response to exercise in humans following acclimatization to extreme altitude. *Acta Physiologica Scandinavica* 154, 499-509.

Sawhney, R.C. Chabra, P.C. and Malhotra, A.S. (1985), Hormone profiles at high altitude in man. *Andrologia* 17, 178-184.

Sawka, M.N. Dennis, R.C. Gonzalez, R.R. Young, A.J. Muza, S.R. Martin, J.W. Wenger, C.B. Francesconi. R.P. Pandolf, K.B. and Valeri, C.R. (1987), Influence of polycythemia on blood volume and thermoregulation during exercise-heat stress. *Journal of Applied Physiology* 62 (3), 912-918.

Sawka, M.N. and Young, A.J. (1989), Acute polycythemia and human performance during exercise and exposure to extreme environments, in, Pandolf, K.B. (ed). *Exercise and Sports Science Review*. Baltimore, MD: Williams and Wilkins, pp 265-293.

Sawka, M.N. Joyner, M.J. Miles, D.S. Robertson, R.J. Spriet, L.L. and Young, A.J. (1996), The use of blood doping as an ergogenic aid. A position stand. *Medicine and Science in Sports and Exercise* 28, pp i-viii.

Schantz, P.G. Sjoberg, B. and Svedenhag, J. (1986), Malate-aspartate and alpha-glycerophosphate shuttle enzyme levels in human skeletal muscle: methodological considerations and effect of endurance training. *Acta Physiologica Scandinavica* 128, 397-407.

Schmidt, R.F. (1980), *Physiologie des Menschen*. Heidelberg: Springer Verlag.

Schmidt, W. (1990), Erythropoietin und Sport-Grundlagen und Meinungen. *Sportmedizin* 41, 304-312.

Schmidt, W. Eckardt, K.U. Hilgendorf, A. Strauch, S. and Bauer, C. (1991), Effects of maximal and submaximal exercise under normoxic and hypoxic conditions on serum erythropoietin level. *International Journal of Sports Medicine* 12 (5), 457-461.

Schmidt, W. Spielvogel, H. Eckardt, K.U. Quintela, A. and Penaloza, R. (1993), Effects of chronic hypoxia and exercise on plasma erythropoietin in high-altitude residents. *Journal of Applied Physiology* 74 (4), 1874-1878.

Schoeffel, R.E. Anderson, S.D. and Altounyan, R.E.C. (1981), Bronchial hyperreactivity in response to inhalation of ultrasonically nebulized solutions of distilled water and saline. *British Medical Journal* 283, 1285-1287.

Schoeller, D.A. and Webb, P. (1984), Five-day comparison of the doubly labeled water method with respiratory gas exchange. *American Journal of Clinical Nutrition* 40, 153-158.

Schoene, R.B. (1982), Control of ventilation in climbers to extreme altitude. *Journal of Applied Physiology* 53, 886-890.

Schoene, R.B. Lahiri, S. Hackett, P.H. Peters, R.M.Jr. Milledge, J.S. Pizzo, C.J. Sarnquist, F.H. Boyer, S.J. Graber, D.J. and Maret, K.H. (1984), Relationship of hypoxic ventilatory response to exercise performance on Mount Everest. *Journal of Applied Physiology* 56 (6), 1478-1483.

Selby, G.B. and Eichner, E.R. (1994), Hematocrit and performance: the effect of endurance training on blood volume. *Seminars in Haematology* 31, 122-127.

Semenza, G.L. (1994), Regulation of erythropoietin production. .New insights into molecular mechanisms of oxygen homeostasis. *Hematology and Oncology Clinics of North America* 8 (5), 863-884.

Sewell, D.A. Gleeson, M. and Blannin, A.K. (1994), Hyperammonaemia in relation to high-intensity exercise duration in man. *European Journal of Applied Physiology and Occupational Physiology* 69, 350-354.

Shephard, R.J. (1966), The oxygen cost of breathing during vigorous exercise . *Quarterly Journal of Experimental Physiology* 51, 336-350.

Shephard, R.J. Bouhlel, E. Vandewalle, H. and Monod, H. (1988), Peak oxygen intake and hypoxia. *International Journal of Sports Medicine* 9, 279-283.

Shephard, R.J. (1992) Problems of high altitude, in Shephard, R.J. and Astrand, P.O. *Endurance in Sport*. London: Blackwell Science Limited, pp 471-478.

Shephard, R.J. and Pang, N.S. (1994), Potential impact of physical activity and sport on the immune system - a brief review. *British Journal of Sports Medicine* 28 (4), 247-255.

Shibanuma, M. Kuroki, T. and Nose, K. (1990), Stimulation by hydrogen peroxide of DNA synthesis, competence family gene expression and phosphorylation of a specific protein in quiescent Balb/3T3 cells. *Oncogene* 5, 1025-1032.

Shivastava, K.K. and Malhotra, M.S. (1974), Effect of adaptation of high altitude on components of blood and urine of lowlanders compared to high altitude natives. *International Journal of Biometeorology* 13, 279-287.

Sime, F. Penaloza, D. Ruiz, L. Gonzalez, N. Covarrubias, E. and Postigo, R. (1974), Hypoxaemia, pulmonary hypertension and low cardiac output in newcomers to low altitude. *Journal of Applied Physiology* 36, 561-565.

Simoneau, J.A. (1995), Adaptation of human skeletal muscle to exercise-training. *International Journal of Obesity* 19 (4), S9-S13.

Simon-Schnass, I. (1994), Risk of oxidative stress during exercise at high altitude, in Sen, C.K. Packer, L. and Hanninen, O. (eds). *Exercise and Oxygen Toxicity*, Amsterdam: Elsevier 1, pp 191-210.

Singh, I. Chohan, I.S. Lal, M. Khanna, P.K. Srivastava, M.C. Nanda, R.B. Lamba, J.S. and Malhotra, M.S. (1977), Effects of high altitude stay on the incidence of common diseases in man. *International Journal of Biometeorology* 21, 93-122.

Siri, W.E. (1956), The gross composition of the body. *Advances in Biological and Medical Physiology* 4, 239-280.

Sjodin, B. and Svedenhag, J. (1994), Assessment of endurance capacity, in Harries, M. Williams, C. Stanish, W. and Micheli, L. (eds). *Oxford Textbook of Sports Medicine*, London: Oxford University Press, pp 172-179.

Smith, J.A. (1995), Exercise, training and red blood cell turnover. *Sports Medicine* 19 (1), 9-31.

Snoke, J.E. Yanan, S. and Bloch, K. (1953), Synthesis of glutathione from γglutamylcysteine. *Journal of Biology and Chemistry* 201, 573-586.

Souba, W.W. (1992), *Glutamine: physiology, biochemistry and nutrition in critical illness*, in Landes, R.G. (ed), Austin.

Spriet, L.L. Gledhill, N. Froses, A.B. Wilkes, D.L. and Meyers, E.C. (1980), The effects of induced erythrocythemia on central circulation and oxygen transport during maximal exercise. *Medicine and Science in Sports and Exercise* 12, 122-123.

Stein, V.T. Baumstark, M.W. Berg, A. and Keul, J. (1991), Lipoprotein (a) - Serum konzentrationen bei Personen mit unterschiedlicher Trainings - anamnese, in, Bernett (ed), *Sport und Medizin - Pro und Contra*, Munich: W.Zuckschwerdt Verlag, pp 530-532.

Stenberg, J. Ekblom, B. and Messin, R. (1966), Hemodynamic response to work at simulated altitude, 4,000 m. *Journal of Applied Physiology* 21, 1589-1594.

Stine, T.A. Levine, B.D. Taylor, S. Schultz, W. and Stray-Gundersen, J. (1992), Quantification of altitude training in the field. *Medicine and Science in Sports and Exercise* 24 (5), Abstract 618

Stone, M.H. Keith, R.E. Kearney, J.T. Fleck, S.J. Wilson, G.D. and Triplett, N.T. (1991), Overtraining: A review of the signs, symptoms and possible causes. *Journal of Applied Sport Science Research* 5 (1), 35-50.

Stoneham, M.D. and Pethybridge, R.J. (1993), Acclimatisation to altitude: Effects on arterial oxygen saturation and pulse rate during prolonged exercise at altitude. *Journal of the Royal Navy Medical Service* 79, 3-9.

Stray-Gundersen, J. Alexander, C. Hochstein, A. deLomos, D. and Levine, B.D. (1992), Failure of red cell volume to increase to altitude exposure in iron deficient runners. *Medicine and Science in Sports and Exercise* 24 (90).

Stray-Gundersen, J. Mordecai, N. and Levine, B.D. (1995), O_2 transport response to altitude training in runners. *Medicine and Science in Sports and Exercise* 27 (5), Abstract 1131.

Stray-Gundersen, J. and Levine, B.D. (1997), "Living high-training high and low" is equivalent to "living high-training low" for sea level performance. *Medicine and Science in Sports and Exercise* 29 (5), Abstract 783.

Sucec, A.A. Hodgdon, J.A. Roy, B.A. and Hazard, A.A. (1996), Time course of acclimatization to altitude (2240M) in female and male runners, and its effects on $\dot{V}O_2$ max and performance. *Medicine and Science in Sports and Exercise* 26 (5), Abstract 417.

Sullivan, M.J. Binkley, P.K. Unverferth, D.V. and Leier, C.V. (1987), Hemodynamic and metabolic responses of the exercising lower limb of humans. *Journal of Laboratory and Clinical Medicine* 110, 145-152.

Sun, S.F. (1985), Epidemiology of hypertension of the Tibetan plateau (Abstract). Second High Altitude Symposium, Qinghai, China (unpublished proceedings)

Sun, S.F. Droma, T.S. Zhuang, J.G. Tao, J.X. Huang, S.Y. McCullough, R.G. McCullough, R.E. Reeves, C.S. Reeves, J.T. and Moore, L.G. (1990), Greater maximal O_2 uptakes and vital capacities in Tibetan than Han residents of Lhasa. *Respiratory Physiology* 79 (2), 151-161.

Surks, M.I. (1966), Metabolism of human serum albumin in man during acute exposure to high altitudes (14,100 ft). *Journal of Clinical Investigations* 45, 1442-1451.

Suslov, F.P. (1994), Basic principles of training at high altitude, in *New studies in athletics, The IAAF Quarterly Magazine for NSA.* Monaco: Multiprint 9 (2), pp 45-49.

Sutton, J.R. Reeves, J.T. Wagner, P.D. Groves, B.M. Cymerman, A. Malconian, M.K. Rock, P.B. Young, P.M. Walter, S.D. and Houston, C.S. (1988), Operation Everest II: oxygen transport during exercise at extreme simulated altitude. *Journal of Applied Physiology* 64 (4), 1309-1321.

Sutton, J.R. (1994), Physiological and clinical consequences of exercise in heat and humidity, in Harries, M. Williams, C. Stanish, W. and Micheli, L. (eds). *Oxford Textbook of Sports Medicine*. London: Oxford University Press, pp 231-238.

Svedenhag, J. Saltin, B. Johansson, C. and Kaijser, L. (1991), Aerobic and anaerobic exercise capacities of elite middle-distance runners after two weeks of training at moderate altitude. *Scandinavian Journal of Medicine and Science in Sports* 1, 205-214.

Szygula, Z. (1990), Erythrocytic system under the influence of physical training and exercise. *Sports Medicine* 10, 181-197.

Taylor, H.L. Buskirk, E. and Henschel, A. (1955), Maximal oxygen intake as an objective measure of cardio-respiratory performance. *Journal of Applied Physiology* 8, 73-80.

Telford, R.D. Graham, K.S. Sutton, J.R. Hahn, A.G. Campbell, D.A. Creighton, S.W. Cunningham, R.B. Davis, P.G. Gore, C.J. Smith, J.A. and Tumilty, D.McA. (1996), Medium altitude training and sea-level performance. *Medicine and Science in Sports and Exercise* 28 (5), Abstract 741.

Terjung, R.L. (1996), Ammonia metabolism in muscle, in Maughan, R.J. and Shirreffs, S.M. (eds). *Biochemistry of Exercise IX.* Champaign, Illinois: Human Kinetics, pp 485-496.

Terrados, N. Melichna, J. Sylven, C. Jansson, E. and Kaijser, L. (1988), Effects of training at simulated altitude on performance and muscle metabolic capacity in competitive road cyclists. *European Journal of Applied Physiology and Occupational Physiology* 57, 203-209.

Terrados, N. Jansson, E. Sylven, C. and Kaijser, L. (1990), Is hypoxia a stimulus for synthesis of oxidative enzymes and myoglobin? *Journal of Applied Physiology* 68 (6), 2369-2372.

Thompson, J.M. Stone, A. Ginsburg, A.D. and Hamilton, P. (1982), O_2 transport during exercise following blood reinfusion. *Journal of Applied Physiology* 53 (5), 1213 - 1219.

Thorstensson, A. (1976), Muscle strength, fibre types and enzyme activities in man. *Acta Physiologica Scandinavica*. Supplement 443.

Tohmeh, J.F. and Cryer, P.E. (1980), Biphasic adrenergic modulation of β-adrenergic receptors in man. *Journal of Clinical Investigations* 65, 836-840.

Torroni, A. Miller, J.A. Moore, L.G. Zamudio, S. Zhuang, J.G. Droma, T. and Wallace, D.C. (1994), Mitochondrial DNA analysis in Tibet-implications for the origin of the Tibetan population and its adaptation to high altitude. *American Journal of Physical Anthropolgy* 93 (2), 189-199.

Tortora, G.J. and Grabowski, S.R. (1996), *Principles of Anatomy and Physiology, Eighth Edition*, in Roesch, B. and Moore, T. (eds). New York: Harper Collins College Publishers, pp 620-622.

Tucker, A. Stager, J.M. and Cordain, L. (1984), Arterial O_2 saturation and maximum O_2 consumption in moderate-altitude runners exposed to sea level and 3,050 m. *Journal of the American Medical Association* 252, 2867-2871.

Tukey, J.W. (1977), *Exploratory Data Analysis*. Reading, Mass: Addison-Wesley.

Uchakin, P. (1995) Immunological adaptations to altitude training, in The third world congress for sport science. Abstract. pp 209-210.

Valentine, J.W. (1978), The evolution of multicellular plants and animals. *Scientific American* 239 (3), 104-117.

Vallier, J.M. Chateau, P. and Guezennec, C.Y. (1996), Effects of physical training in a hypobaric chamber on the physical performance of competitive triathletes. *European Journal of Applied Physiology and Occupational Physiology* 73, 471-478.

Vanderwalle, H. Peres, G. and Monod, H. (1987), Standard anaerobic exercise tests. *Sports Medicine* 4, 268-289.

Vanzetti, G.J. (1966), An azide-methemoglobin method for hemoglobin determination in blood. *Journal of Laboratory and Clinical Medicine* 67 (1), 116-126.

Victor, R.G. Bertocci, L.A. Pryor, S.L. and Nunnally, R.L. (1988), Sympathetic nerve discharge is coupled to muscle cell pH during exercise in humans. *Journal of Clinical Investigation* 82, 1301-1305.

Vogel, J.A. Hansen, J.E. and Harris, C.W. (1967), Cardiovascular responses in man during exhaustive work at sea level and high altitude. *Journal of Applied Physiology* 23, 531-539.

Vogel, J.A. Hartley, L.H. and Cruz, J.C. (1974), Cardiac output during exercise in altitude natives at sea level and high altitude. *Journal of Applied Physiology* 36, 173-176.

Voors, A.C.W. and Johnson, W.D. (1979), Altitude and arteriosclerotic heart disease mortality in white residents of 99 of the 100 largest cities in the United States. *Journal of Chronic Disorders* 32, 157-162.

Wagemakers, A.J.M. Schepens, J.T.G. and Veerkamp, J.H. (1984), Effect of starvation and exercise on actual and total activity of the branched-chain 2-oxoacid dehydrogenase complex in rat tissues. *Biochemistry Journal* 223, 815-821.

Wagenmakers, A.J.M. (1992), Amino acid metabolism, muscular fatigue and muscle wasting. Speculations on adaptations at high altitude. *International Journal of Sports Medicine* 13 (1), S110-S113.

Wagner, P.D. Saltzman, H.A. and West, J.B. (1974), Measurement of continuous distributions of ventilation-perfusion ratios: theory. *Journal of Applied Physiology* 36, 588-599.

Wagner, P.D. Gale, G.E. Moon, R.E. Torre-Bueno, J.R. Stolp, B.W. and Saltzman, H.A. (1986), Pulmonary gas exchange in humans exercising at sea level and simulated altitude *Journal of Applied Physiology* 61 (1), 260-270.

Wagner, P.D. Sutton, J.R. Reeves, J.T. Cymerman, A. Groves, B.M. and Malconian, M.K. (1987), Operation Everest II: pulmonary gas exchange during a simulated ascent of Mt. Everest. *Journal of Applied Physiology* 63, 2348-2359.

Ward, M.P. Milledge, J.S. and West, J.B. (1995) *High altitude medicine and physiology. Second edition.* London: Chapman and Hall Medical.

Warnick, R. Benderson, J.M. and Abers, J.J. (1982), Dextran sulfate-Mg^{2+} precipitation procedure for quantification of high-density lipoprotein cholesterol. *Clinical Chemistry* 28, 1379-1388.

Wasserman, K. and McIlroy, M.B. (1964), Detecting the threshold of anaerobic metabolism in cardiac patients during exercise. *American Journal of Cardiology* 14, 844-852.

Wasserman, K. Whipp, B.J. Koyal, S. and Beaver, W. (1973), Anaerobic threshold and respiratory gas exchange during exercise. *Journal of Applied Physiology* 35, 236-243.

Wasserman, K. (1987), *Principles of exercise testing and interpretation.* Philadelphia: Lea and Febiger.

Wasserman, K., Hansen, J.E., Sue, D.Y., Whipp, B.J. and Casaburi, R. (1994) *Principles of Exercise Testing and Interpretation.* Philadelphia: Lea and Febiger.

Weight, L.M. Klein, M. Noakes, T.D. and Jacobs, P. (1992), Sports anemia - A real or apparent phenomenon in endurance-trained athletes. *International Journal of Sports Medicine* 13, 344-347.

Weil, J.V. Jamieson, G. Brown, D.W. and Grover, R.F. (1968), The red cell mass-arterial oxygen relationship in normal man. *The Journal of Clinical Investigation* 47, 1627-1639.

Welch, H.G. Petersen, F.B. Graham, T. Klausen, K. and Secher, N. (1977), Effects of hyperoxia on leg blood flow and metabolism during exercise. *Journal of Applied Physiology* 42, 385-390.

Welch, H.G. (1987), Effects of hypoxia and hyperoxia on human performance. *Exercise Sports Science Reviews* 15, 191-221.

West, J.B. (1962), Diffusing capacity of the lung for carbon monoxide at high altitude. *Journal of Applied Physiology* 17, 421-426.

West, J.B. (1983), Climbing Mt Everest without supplementary oxygen: an analysis of maximal exercise during extreme hypoxia. *Respiratory Physiology* 52, 265-279.

West, J.B. Lahiri, S. and Maret, K.H. (1983b), Barometric pressures at extreme altitudes on Mt Everest: physiological significance. *Journal of Applied Physiology* 54, 118-119.

West, J.B. (1986), Lactate during exercise at extreme altitude. *Federation Proceedings* 45, 2953-2957.

Westerterp, K.R. Kayser, B. Brouns, F. Herry, J.P. and Saris, W.H. (1992), Energy expenditure climbing Mt Everest. *Journal of Applied Physiology* 72, 1815-1819.

Westerterp, K.R. Kayser, B. Wouters, L. Le Trong, J.L. and Richalet, J.P. (1994), Energy balance at high altitude: 6542m. *Journal of Applied Physiology* 77 (2), 862-866.

Whipp, B.J. and Ward, S.A. (1994), Respiratory responses of athletes to exercise, in Harries, M. Williams, C. Stanish, W. and Micheli, L. (eds). *Oxford Textbook of Sports Medicine*. London: Oxford University Press, pp 13-27, 1994.

Whitten, B.K. and Janoski, A.H. (1969), Effects of high altitude and diet on lipid components of human serum. *Federation Proceedings* 28 (3), 983-986.

Williams, M.H. Wesseldine, S. Somma, T. and Schuster, R. (1981), The effect of induced erythrocythemia upon 5-mile treadmill run time. *Medicine and Science in Sports and Exercise* 13 (3), 169-175.

Williams, M.H. (1992), Bicarbonate loading. *Sports Science Exchange* 4 (36).

Williams, C. (1995), Haemoglobin - is more better? *Nephrology Dialysis Transplantation* 10 (2), 48-55.

Wilmore, J.H. and Costill, D.L. (1994), *Physiology of Sport and Exercise*. Champaign, Illinois: Human Kinetics, pp 221.

Windmueller, H.G. and Spaeth, A.E. (1974), Uptake and metabolism of plasma glutamine by the small intestine. *Journal of Biology and Chemistry* 249, 5070-5079.

Winslow, R.M. and Monge, C.C. (1987), *Hypoxia, polycythemia and chronic mountain sickness*. Baltimore: John Hopkins University Press.

Winslow, R.M. Chapman, K.W. Gibson, C.C. Samaja, M. Monge, C.C. Goldwasser, E. Sherpa, M. Blume, F.D. and Santolaya, R. (1989), Different hematologic responses to hypoxia in Sherpas and Quechua Indians. *Journal of Applied Physiology* 66 (4), 1561-1569.

Witte, D.L. (1991), Can serum ferritin be effectively interpreted in the presence of the acute phase response? *Clinical Chemistry* 37, 484-485.

Wolfel, E.E. Groves, B.M. Brooks, G.A. Butterfield, G.E. Mazzeo, R.S. Moore, L.G. Sutton, J.R. Bender, P.R. Dahms, T.E. McCullough, R.E. McCullough, R.G. Huang, S.Y. Sun, S.F. Grover, R.F. Hultgren, H.N. and Reeves, J.T. (1991), Oxygen transport during steady-state submaximal exercise in chronic hypoxia. *Journal of Applied Physiology* 70, 1129-1136.

Wolski, L.A. McKenzie, D.C. and Wenger, H.A. (1996), Altitude training for improvements in sea level performance. Is there scientific evidence for benefit? *Sports Medicine* 22 (4), 251-263.

Young, A. (1990), Energy substrate utilization during exercise in extreme environments. *Exercise Sports Science Reviews* 18, 65-118.

Young, A.J. Wright, J. Knapik, J. and Cymerman, A. (1980), Skeletal muscle strength during exposure to hypobaric hypoxia. *Medicine and Science in Sports and Exercise* 12 (5), 330-335.

Young, A.J. Cymerman, A. and Pandolf, K.B. (1982), Differentiated ratings of perceived exertion are influenced by high altitude exposure. *Medicine and Science in Sports and Exercise* 14, 223-228.

Young, A.J. Evans, W.J. Cymerman, A. Pandolf, K.B. Knapik, J.J. and Maher, J.T. (1982), Sparing effect of chronic high-altitude exposure on muscle glycogen utilisation. *Journal of Applied Physiology* 52, 857-862.

Young, A.J. Evans, W.J. Fisher, E.C. Sharp, R.L. Costill, D.L. and Mahr, J.T. (1984), Skeletal muscle metabolism of sea-level natives following short-term high-altitude residence. *European Journal of Applied Physiology and Occupational Physiology* 52 (4), 463-466.

Young, A.J. and Young, P.M. (1988) Human acclimatization to high terrestrial altitude, in Pandolf, K.B. et al (eds). *Human performance physiology and environmental medicine at terrestrial extremes*. Indianapolis: Benchmark Press, pp 497-543.

Young, A.J. Young, P.M. McCullough, R.E. Moore, L.G. Cymerman, A. and Reeves, J.T. (1991), Effect of beta-adrenergic blockade on plasma lactate concentration during exercise at high altitude. *European Journal of Applied Physiology and Occupational Physiology* 63, 315-322.

Young, A.J. Sawka, M.N. Muza, S.R. Boushel, R. Lyons, T. Rock, P.B. Freund, B.J. Waters, R. Cymerman, A. Pandolf, K.B. and Valeri, C.R. (1996), Effects of erythrocyte infusion on VO_2 Max at high altitude. *Journal of Applied Physiology* 81 (1), 252-259.

Young, P.M. Rock, P.B. Fulco, C.S. Trad, L.A. Forte, V.A. and Cymerman, A. (1987), Altitude acclimatization attenuates plasma ammonia accumulation during submaximal exercise. *Journal of Applied Physiology* 63 (2), 758-764.

Young, P.M. Rose, M.S. Sutton, J.R. Green, H.J. Cymerman, A. and Houston, C.S. (1989), Operation Everest II: plasma lipid and hormonal responses during a simulated ascent of Mt. Everest. *Journal of Applied Physiology* 66 (3), 1430-1435.

Young, P.M. Sutton, J.R. Green, H.J. Reeves, J.T. Rock, P.B. Houston, C.S. and Cymerman, A. (1992), Operation Everest II: metabolic and hormonal responses to incremental exercise to exhaustion. *Journal of Applied Physiology* 73 (6), 2574-2579.

Young, S.J. (1986), The clinical psychopharmacology of tryptophan, in Wurtman, R.J. and Wurtman, J.J. (eds). *Nutrition and the Brain.* New York: Raven Press, pp 49-88.

Zhuang, J. Droma, T. Sutton, J.R. McCullough, R.E. McCullough, R.G. Groves, B.M. Rapmund, G. Janes, C. Sun, S. and Moore, L.G. (1993a), Autonomic regulation of heart rate response to exercise in Tibetan and Han residents of Lhasa (3,658 m). *Journal of Applied Physiology* 75 (5), 1968-1973.

Zhuang, J. Droma, T. Sun, S. Janes, C. McCullough, R.E. McCullough, R.G. Cymerman, A. Huang, S.Y. Reeves, J.T. and Moore, L.G. (1993b), Hypoxic ventilatory responsiveness in Tibetan compared with Han residents of 3,658 m. *Journal of Applied Physiology* 74, 303-311.

Zuntz, N. Loewy, A. Muller, F. and Caspari, W. (1981), Atmospheric pressure at high altitudes, in *Hohenklima und Bergwanderungen in ihrer Wirkung auf den Menschen*, Bong, Berlin, 1906. Translated in High altitude physiology (West, J.B. ed), Stroudsbourg, Pennsylvania: Hutchinson Ross Publishing Company, pp 37-39.

APPENDICES

APPENDIX A: GENERAL HEALTH QUESTIONNAIRE

BRITISH OLYMPIC MEDICAL CENTRE

PHYSIOLOGY LABORATORY TESTING

PLEASE PRINT

Surname		First Name	
Date of Birth		Date of Visit	
Address			
Postcode			
Tel No (Home)		Tel No (Work)	
Coach		Coach Tel No	

GENERAL HEALTH QUESTIONNAIRE

Please mark appropriate box with a √

Do you , or have you ever suffered from	YES	NO
Diabetes?		
Asthma?		
Epilepsy?		
Have you ever had pains in your chest or heart?		
Do you often feel faint or have spell of severe dizziness?		
Have you ever had high blood pressure?		
Has your doctor ever told you that you have a bone or joint problem that might be made worse with severe exercise (such as laboratory testing)?		
In the past week, have you suffered from any illness which required you to be in bed or off work for one day or more?		
Is there a good physical reason not mentioned here why you should not carry out laboratory testing?		
Do you smoke?		

Please give details of any training you did yestersday.

_____

_____

_____

IMPORTANT:

Please provide any further information concerning <u>any</u> condition /complaints which you are suffering from and <u>any</u> medication which you may be taking by prescription or otherwise.

_____

_____

_____

_____ _____

Signature of Participant Date

Please continue on next page

APPENDIX B: PERFORMANCE PROGRESS REPORT

ALBUQUERQUE ALTITUDE STUDY
ATHLETE: Mr X

Dear **Mr X,**
Thank you for having participated in the physiological testing. I have summarised the major results which are presented in both a descriptive and graphical format. An explanation of the scientific terminology employed is also presented.

ALTITUDE - WHAT IS IT?

In general, you create what is termed a "hypoxic stimulus" when you train. In short, you are depriving your muscles of oxygen. In response to this and over a prolonged period of time, your physiology adapts to this stimulus and you are able to transport and use the available oxygen more efficiently. Altitude training increases such a stimulus, imposing a further oxygen demand on your muscles. In theory, the rate of adaptation should increase and you should become faster and fitter than before the trip!

ALTITUDE DATA

- **Lying supine heart rate** remained reasonably stable even after the track sessions, and indicated that your body was sufficiently coping with the demands placed upon it.
- **The blood pressure** response was perfectly normal during your stay at altitude
- **Packed cell volume** reached a value of **0.47 $L.L^{-1}$** on the final day of testing at altitude. This equates to a **7%** increase in comparison to pre - altitude values **0.44 $L.L^{-1}$.** These values compare well with the male group mean (**0.48 $L.L^{-1}$**). Other studies have reported 7% increases in PCV after 3 weeks of training at 1900 metres.
- Haemoglobin values ranged from **14.8 - 16.2g/dl**. Again, compare yourself to other **males at altitude (mean of 15.7g/dl).** In general, altitude studies have reported individual increases in Hb of 1 - 4% after a 3 week stay at a moderate altitude (1300 - 2500 metres). Remember, we stayed at approximately 1,500 meters and during training runs, we climbed to 2000 metres (Track is located at 1,500 metres).
- **The laboratory session** data suggests that the physiological load was reduced at altitude (see accompanying graph). This is an intriguing observation as one would expect the opposite with a reduction in the partial pressure of oxygen!
- **The track session** - Rep times were on average 1 secs slower at altitude even though environmental conditions were ideal (Slight breeze, 28°C/2% Relative humidity). Lactate concentrations were slightly higher.

POST ALTITUDE DATA

- Changes in both body mass and blood pressure pre versus post altitude are insignificant.
- Hemoblobin values have significantly increased by **14%** approximately 3 weeks post altitude
- PCV increased by **11%** which in combination with the hemoblobin increase is a powerful adaptation and potential performance improver!
- No real changes occurred regarding your lung function values and provide further support that they are emminently designed to cope with the heavy demands of exercise
- **Laboratory data** indicated lower heart rates, 23% lower blood lactates yet similar oxygen uptake values (see accompanying graph).
- **Track data** indicated a 1% increase in running speed in comparison to the pre-altitude track session **at lower lactates.** The lower concentrations of lactic acid allow you to run faster, as evidenced by higher heart rates.

In summary, it would appear that physiological performance has improved following 4 weeks altitude exposure (1500 - 2000 metres)
A given running speed now represents a reduced physiological workload!

A definitive answer relating to the effectiveness of altitude training will be communicated when I have completed a statistical analysis of the GROUP results

If you have any questions regarding the interpretation of the results please contact me. Have a good season!

D. Bailey

Damian M. Bailey
Research Physiologist BOMC

EXPLANATION OF TERMS

- **Hb (g/dl)** - This is a measurement of the amount of haemoglobin per 100 millilitres of blood. Haemoglobin binds to oxygen and up to as much as a billion molecules of oxygen can be transported by a single red blood cell! Normal sedentary values range between
  14 - 18g/dl for males and 12 - 16 g/dl for females.

- **Serum ferritin** - an indication of your body's iron stores. Normal values for males are 20-300 ng/ml and females, 10-300 ng/ml.

  Cholesterol - a substance which is implicated in the aggregation of fatty deposits in blood vessel walls termed arteriosclerosis. The "desired" concentration of cholesterol for males and females are is < 5.2 $mmol.L^{-1}$.

- **Plasma glutamine** - an amino acid which is implicated in the control of immune function. A decrease *may* signify immunosuppression which may increase your susceptibility to developing a respiratory infection. "Overtrained" athletes have been demonstrated to suffer from chronically depressed values. Normal values for healthy male and female athletes are typically between 600-700 μM.

- **BP(mm/Hg)** - This refers to your blood pressure. A normal value at rest is 120/80mmHg

- **FVC ($L.min^{-1}$)** - The Forced Vital Capacity is the volume of air that can be exhaled from a position of full inspiration , as rapidly and completely as possible.

- **RPE** - The Rating of Perceived Exertion allows **you** to subjectively interpret the level of exercise intensity on a scale of 6 -20

- **Lactate ($mmol.L^{-1}$)** - Is a concentration of lactic acid produced by the muscle. In general, for an endurance athlete, the lower the concentration the better!

- **$\dot{V}O_{2max}$ (ml.kg.$^{-1}$min^{-1})** - A respiratory measurement of oxygen uptake. As your aerobic adaptations improve through training, your $\dot{V}O_{2max}$ may increase. Typical values for elite endurance athletes (5,000 to 10,000 metres specialists at the British Olympic Medical Centre are 75-90 ml.kg.$^{-1}$min^{-1} for males and 60-75 ml.kg.$^{-1}$min^{-1} for females).

RESULTS DATA SHEET 1:
ATHLETE: Mr X

Age (Years): 18
Stature (m): 1.85
Body Fat (%): 6.0

DESCRIPTIVE DATA

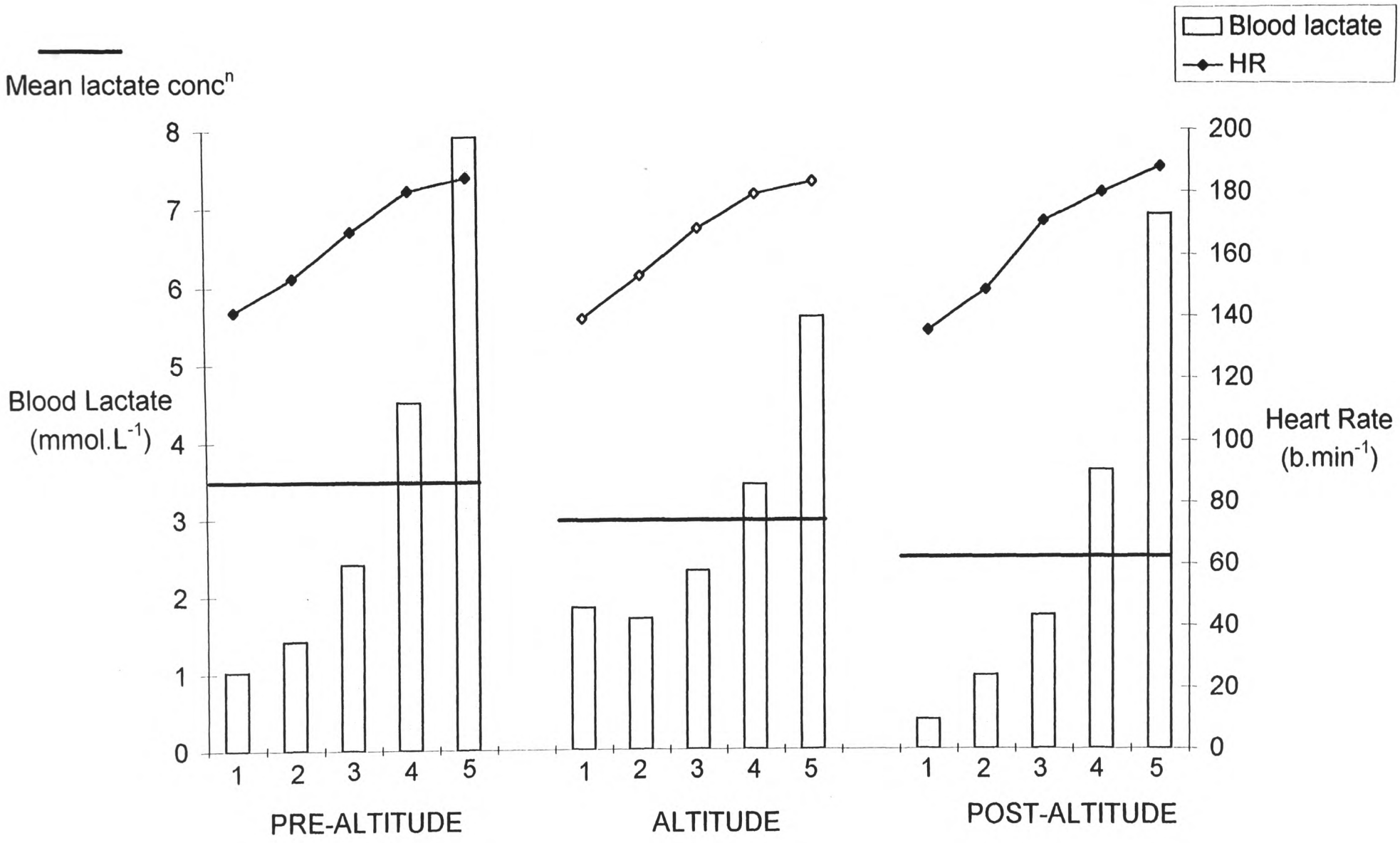
Variable:	PRE	ALTITUDE	POST
Body mass (kgs)	69.95	70.0 ± 1.0	69.80
BP (mmHg)	126/78	122 ± 3/62 ± 2	120/68
Hb (g/dl)	13.8	14.7 ± 0.3	15.8
PCV (L/L)	0.44	0.47 ± 0.02	0.49
MVV ($L.min^{-1}$)	202.5	.....	203.2
% predict	121	.....	122
FVC ($L.min^{-1}$)	6.09	.....	6.18
% predict	105	.....	106
FEV_1 ($L.min^{-1}$)	5.18	.....	5.23
%predict	106	.....	107
FEV_1 /FVC ($L.min^{-1}$)	85.1	.....	84.6
% predict	101	.....	100

LABORATORY DATA
(Based on mean values during 5 incremental speeds)

Variable:	PRE	ALTITUDE	POST
HR ($b.min^{-1}$)	173	165	165
Sweat Rate ($ml.min^{-1}$)	17.9	59.5	19.0
RPE	14	14	14
Lactate ($mmol.L^{-1}$)	3.46	2.99	2.72
$\dot{V}O_2$ ($L.min^{-1}$)	3.82	.....	3.89
$\dot{V}_E$ ($L.min^{-1}$)	117.6	.....	113.1
RER	0.99	.....	0.97

TRACK DATA
(Based on mean values during 4 x 1000ms / 2 mins recovery)

Variable:	PRE	ALTITUDE	POST
Rep Time (Mins/Secs)	2:58	2:59	2:57
Lactate ($mmol.L^{-1}$)	7.94	8.52	7.45
Mean HR ($b.min^{-1}$)	183	181	187
Peak HR ($b.min^{-1}$)	185	183	188
Recovery HR ($b.min^{-1}$)	177	175	180
RPE	14	15	14



Physiological Responses During a Submaximal Treadmill Test at Sea-Level and Altitude (Subject X)

APPENDIX C: TRAINING DIARY

NAME:
DATE:

DAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY	SUNDAY
AM SESSION **Characteristics:** **Distance (km):** **HEART RATE**							
PM SESSION **Characteristics:** **Distance (km):** **HEART RATE**							
				TOTAL	**WEEKLY**	**MILEAGE:**	

APPENDIX D: MEASUREMENT OF BODY FAT USING ANTHROPOMETRIC CALIPERS

LOCATION OF THE SKINFOLD SITE

Biceps

The anterior surface of the biceps midway between the anterior auxiliary fold and the antecubital fossa. The elbow should be extended and the arm relaxed.

Triceps

A vertical fold on the posterior midline of the upper arm, over the triceps muscle, halfway between the acromion process and olecranon process. The elbow should be extended and the arm relaxed.

Subscapular

The fold is measured on the diagonal line coming from the vertebral border to between 1 and 2 cm from the inferior angle of the scapulae.

Suprailiac

A diagonal fold above the crest of the ilium at the point where an imaginary line would descend from the anterior auxiliary line.

CALCULATION OF BODY FAT

Body fat was determined indirectly using a modification of the original regression equations developed by Durnin and Womersley (1974). These equations were developed and validated at the British Olympic Medical Centre, UK using elite athletes by Mr. B.Carpenter (unpublished observations). Age was included as a variable to avoid the previous changes in estimates of body fat content between adjacent age groups. Body density (BD) was determined using the following equations:

$$BD = 1.1576 - (0.0657 \log_{ss}) - [0.00033 \times Age\ (Years)] - Female\ subjects$$

$$BD = 1.1785 - (0.0657 \log_{ss}) - [0.00049 \times Age\ (Years)] - Male\ subjects$$

where:
$_{ss}$: sum of skinfolds (mean value)

Percent body fat was estimated using the Siri (1956) equation:

$$Body\ fat\ (\%) = \frac{495}{BD} - 450$$

APPENDIX E: CIRCADIAN RHYTHMS: PHYSIOLOGICAL IMPLICATIONS AT REST AND DURING SUBMAXIMAL EXERCISE

AIMS

To evaluate the physiological implications of circadian rhythms at rest and during physical exercise.

METHODS

[1] Two active male subjects reported to the laboratory at 0900 h following an overnight fast and were instructed to refrain from eating or drinking for the duration of the experimental period. Arterialised capillary blood was obtained from a hyperaemic earlobe in the seated position which was subsequently analysed for whole blood lactate ($[La^-]_B$). A medical thermometer was inserted sublingually for the determination of body temperature and resting heart rate (HR) was measured using bipolar 3 lead electrocardiography. This was followed by a standardised treadmill run which lasted 4 minutes at a velocity that elicited 70% of each subject's age predicted maximal heart rate [220 $b.min^{-1}$ - Age (Years)]. A second earlobe blood sample was assayed for $[La^-]_B$. This procedure was repeated on an hourly basis with the final measurements at 1700 h.

[2] The same two subjects reported to the laboratory at 10 00h. Earlobe blood samples were obtained in the seated position at various times throughout the day and subsequently assayed for haemoglobin (Hb) and haematocrit (Hct) concentration. Subjects refrained from eating or drinking during the experimental period.

RESULTS

Mean values for selected cardiovascular and metabolic data are summarised in Table E_1 and Figure E_1:

Table E_1 Changes in Body Temperature and Whole Blood Lactate Concentration ($[La^-]_B$) (n = 2)

Time of Day	Resting Oral Temp (°C)	Resting HR ($bt.min^{-1}$)	Resting $[La^-]_B$ ($mmol.L^{-1}$)	Exercise $[La^-]_B$ ($mmol.L^{-1}$)
09:00	36.1	62	0.67	1.90
10:00	36.1	60	0.75	2.40
11:00	36.2	62	0.68	1.94
12:00	36.5	56	0.80	2.05
13:00	36.7	60	0.78	2.09
14:00	36.7	63	0.92	2.27
15:00	36.6	63	0.68	2.08
16:00	36.7	61	0.92	2.00
17:00	36.8	65	0.88	1.95
Range:	36.1 - 36.8	56 - 65	0.67 - 0.92	1.90 - 2.40

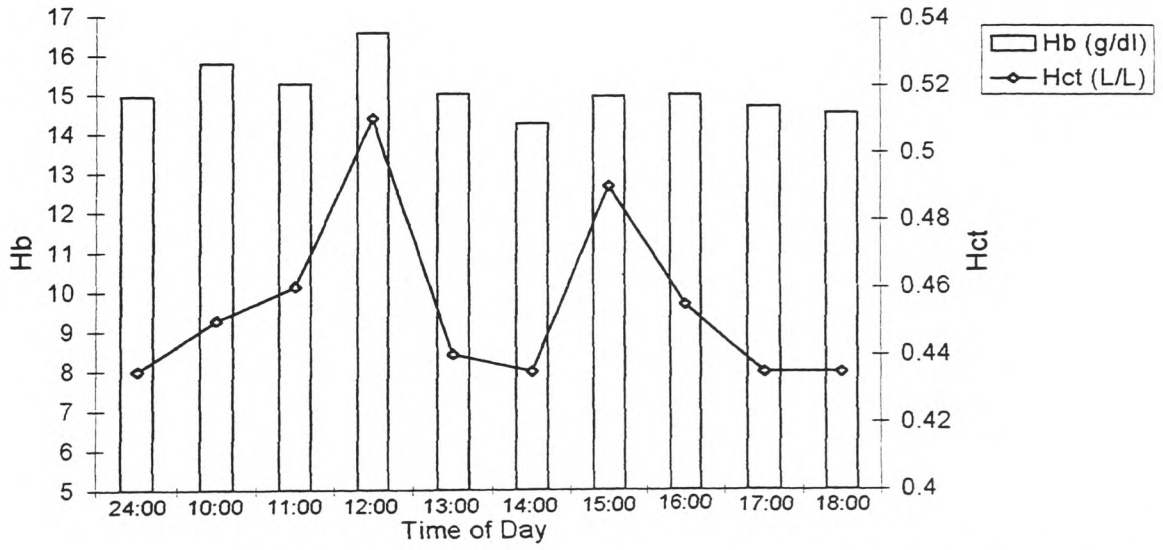


Figure E_1 Time of Day Effects on Resting Haemoglobin (Hb) and Haematocrit (Hct) Concentration (n = 2)
Hb range: 14.3 to 16.6 g/dl
Hct range: 0.44 to 0.51 L/L

DISCUSSION

Circadian rhythms have been shown to influence biological function and have a profound effect on physical performance (Reilly, 1994). Previous researchers have identified significant changes in heart rate (Reilly and Brooks, 1982), body temperature (Reilly and Brooks, 1986) and exercise performance (Baxter and Reilly, 1983) at different times during the day. The data in the present study also highlights the circadian variation of Hb, Hct and $[La^-]_B$, dependent variables which, to this author's knowledge, do not appear to have been investigated in the literature despite their widespread use. Failure to account for the normal biological variation of a physiological parameter would undoubtedly influence the interpretation of scientific data. Whilst changes in a dependent variable due to a treatment effect may be of statistical significance, the question arises as to whether the change represents any physiological significance (Fraser and Fogarty, 1989).

APPENDIX F: THE EFFECTS OF THE MENSTRUAL CYCLE ON ENDURANCE PERFORMANCE: A CASE STUDY

AIM

To determine the effects of the menstrual cycle on physiological indices of laboratory based endurance performance in a sedentary female.

METHOD

A sedentary female subject was habituated to laboratory testing procedures and instructed to refrain from any physical exercise and maintain a normal diet throughout the duration of the experimental period. Subject anthoprometric data are summarised in Table F_1. The subject reported to the laboratory at 0900 h following an overnight fast and performed a standardised treadmill protocol (Figure K_1/Appendix K) for the determination of mean values for HR, $\dot{V}_E$, $\dot{V}O_2$ and $[La^-]_B$. This procedure was repeated every other day during the course of the menstrual cycle.

Table F_1 Anthropometric Data

Variable	Subject GR
Age (Yr)	23
Stature (m)	1.63
Body Mass (kg)	54.8
BMI (kg/m^2)	20.6
Body Fat (%)	20
$\dot{V}O_{2max}$ ($L.min^{-1}$)	2.07
$\dot{V}O_{2max}$ ($ml.kg.^{-1}min.^{-1}$)	37.8

RESULTS

A summary of the findings is illustrated below in Table F_2. Data represent mean values that were obtained during the menstrual, proliferative and secretory phases of the menstrual cycle.

Table F_2 Changes in Selected Cardiorespiratory and Metabolic Parameters During the Menstrual Cycle (n = 1)

Dependent Variable	Menstrual Phase (Days 1 - 5)	Proliferative Phase (Days 6 - 14)	Secretory Phase (Days 15 - 28)
$[La^-]_B$ (mmol.L^{-1})	3.57	3.63	3.80
$\dot{V}_E$ (L.min^{-1})	34.2	35.6	35.6
$\dot{V}O_2$ (L.min^{-1})	0.84	0.88	0.82
HR (bt.min^{-1})	121	124	123

DISCUSSION

The present study did not identify any changes in any of the physiological indices of endurance performance at various stages of the menstrual cycle. However, previous research has demonstrated that the oestradiol and progesterone responses vary during the menstrual cycle which may influence the metabolic response to physical exercise (Howlett and Grossman, 1994). Physical performance has also been shown to be affected. Brooks-Gunn et al. (1986) demonstrated that swimming performance times improved during the flow phase of the menstrual cycle. Whilst the present findings are equivocal, these data highlight the potential contaminating effects of the female androgens on physiological performance.

APPENDIX G: VALIDATION OF HEART RATE MEASUREMENTS

HEART RATE MONITOR		
Pulse Meter* ($bt.min^{-1}$)	**Polar Vantage NV™ ($bt.min^{-1}$)**	**Rigel ECG ($bt.min^{-1}$)**
40	40	41
60	61	62
80	80	80
100	101	100
120	121	121
140	140	141
160	160	160
180	181	180
200	200	200

*: Rigel ECG Stimulator, 202 (Graseby Medical Limited, UK)
No significant differences between heart rate monitors during trials ($P > 0.05$)

APPENDIX H: RATINGS OF PERCEIVED EXERTION (Borg, 1973)

6

7 **Very, very light**

8

9 **Very light**

10

11 **Fairly light**

12

13 **Somewhat hard**

14

15 **Hard**

16

17 **Very hard**

18

19 **Very, very hard**

20

APPENDIX I: VALIDATION OF ON-LINE DETERMINATION OF OXYGEN UPTAKE

AIMS

To validate two on-line respiratory analysers: [1] Jaeger EOS-Sprint (Market Harborough, UK) and [2] MedGraphics^{R} Cardiopulmonary exercise systems CPX/D, Cardiokinetics, UK) with an off-line Douglas Bag reference system.

METHODS

Two elite male subjects reported to a temperature controlled laboratory (21 ± 1°C) following an overnight fast at 0900 h on Monday, Wednesday and Friday. Both subjects were familiar with laboratory testing procedures. They were randomnly assigned to either the Jaeger EOS-Sprint, MedGraphics^{R} Cardiopulmonary exercise systems CPX/D or a Douglas Bag system for the measurement of maximal oxygen uptake ($\dot{V}O_{2max}$) according to a standard method developed at the British Olympic Medical Centre (UK).

The protocol consisted of a 5 minute warm-up at 12.6 km.hr^{-1}. Thereafter, the treadmill velocity was increased by 1.1 km.hr^{-1} every 60 seconds. The final stage was completed at 21.4 km.hr^{-1} at a 1% gradient. The total test duration was 14 minutes exactly. On-line respiratory parameters were printed continously during 30 s intervals. Expired gas samples were collected between minutes; [1] 4 - 5 [2] 9 - 10 and [3] 13 - 14 and subsequently analysed for minute ventilation ($\dot{V}_E$), oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$). A summary of the results expressed as a mean value is outlined below in Table I_1:

RESULTS

Table I_1 Validation of On-Line Gas Analysis

	$\dot{V}_E$ (L.min^{-1} STPD)			$\dot{V}O_2$ (L.min^{-1} STPD)		
Sample	**A**	**B**	**C**	**A**	**B**	**C**
1	*72*	74	73	*2.78*	2.80	3.14
2	*94*	92	91	*3.32*	3.30	3.51
3	*111*	110	108	*3.74*	3.82	3.86

A: Douglas Bag System
B: Jaeger EOS-Sprint (Market Harborough, UK)
C: MedGraphics^{R} Cardiopulmonary exercise systems CPX/D, (Cardiokinetics, UK)

DISCUSSION

The data would suggest that the performance of the on-line respiratory analysers was both accurate and precise. However, the lack of sufficient degrees of freedom precluded the application of any statistical analysis. Further research with a larger sample size is warranted to validate on-line respiratory gas analysis.

APPENDIX J: DETERMINATION OF THE CRITICAL DIFFERENCE FOR SELECTED PHYSIOLOGICAL PARAMETERS

INTRODUCTION AND AIMS

Changes in physiological results are caused by analytical imprecision (Buttner et al 1979) and biological or within subject variation (Harris, 1970). Whilst the concentration of a metabolite measured in response to exercise or changes in environmental pressure may change significantly ($P < 0.05$), the magnitude of the change may be too small to be of any physiological significance. Differentiation between statistical and physiological significance is therefore important in any scientific experimentation to determine whether any *real* changes have occurred.

This can be determined by calculating the critical difference for any number of dependent variables (Costongs et al 1985):

$$Critical\ difference = K.\ \sqrt{(CV_a^2 + Cv_w^2)}$$

where:

K - factor dependent on the probability level slected (2.77 at $P < 0.05$)
Cv_a - coefficient of anaytical variation
Cv_w - coefficient of within subject variation

Thus the present investigation calculated the critical difference of a variety of metabolic and cardiorespiratory parameters that were to be measured during Studies 1 and 2.

METHOD

Calculation of analytical variation

[1] Haematological measurements: Fifteen samples of quality control sera were injected into a Refletron and an Analox Champion PLM5 for the determination of Cv_a serum urea and Cv_a $[La^-]_B$ respectively. Cv_a Hb was determined by performing 15 repeated measurements of an optical interference filter.

[2] Respiratory measurements: An investigator was randomnly assigned to manually pumping either 120, 150 or 180 $L.min^{-1}$ of ambient air (relative humidity = 45%, temperature = 21°C) via a 3 litre syringe into either a Jaeger EOS-Sprint or a MedGraphics^R CPX/D on-line respiratory gas analyser to validate the performance of the pneumotachograph system. The upper limits of this procedure were determined in a separate study which demonstrated that twenty elite distance runners were capable of achieving a maximum $\dot{V}_E$ of 176 ± 14 $L.min^{-1}$ (unpublished data, British Olympic Medical

Centre, UK). The pumping action of the syringe was timed using a calibrated metronome. This procedure was performed a total of ten times.

Calculation of biological variation

Cv_w was calculated during an experimental procedure following habituation which is outlined in Appendix K. Calculation of the Cv_w was determined by:

$$Cv_w(\%) = Total\ variation\ (\%) - Cv_a(\%)$$

RESULTS

Table J_1 summarises the Cv_a and the Cv_w and subsequent calculation of the critical difference for a variety of physiological variables.

Table J_1 The Critical Difference of Selected Physiological Parameters

Variable	$[La^-]_B$	Serum urea	Hb	$\dot{V}_E$	$\dot{V}O_2$	HR
Cv_a (%)	0.05	2.6	0.8	0.1	0.05	0.1
Cv_w (%)	14.95	9.0	11.6	5.0	6.55	5.4
Total variation (%)	15.0	11.6	12.4	5.1	6.60	5.5
Critical Diff (%)	*41.4*	*25.9*	*32.2*	*13.9*	*18.1*	*15.0*

DISCUSSION

This research did not permit rigorous statistical treatment of the data because of the restricted degrees of freedom. However, within the constructs of the study, a repeated measures design highlighted the magnitude of variation.

APPENDIX K: PHYSIOLOGICAL IMPLICATIONS OF HABITUATION ON TREADMILL PERFORMANCE

INTRODUCTION AND AIMS

To this author's knowledge, only one study has quantified the effects of habituation on treadmill performance in elite distance runners (Saltin et al 1995). A group of Kenyan distance runners performed 7 "practice" sessions to habituate to treadmill running prior to their involvement in a major experiment. Each practice session consisted of 5 minutes each at 6, 8, 10, 12, 14 and 16 $km.hr^{-1}$ at a constant grade of 2.8%. A brief summary of their findings is illustrated in Table K_1.

Table K_1 Effects of Habituation on Treadmill Performance (Saltin et al 1995)

Practice Session	$[La^-]_B$ ($mmol.L^{-1}$)	Mean Heart Rate ($bt.min^{-1}$)	Mean $\dot{V}O_2$ ($L.min^{-1}$)
2	4.9	180	2.0
7	2.6	165	1.8
Change (%)	*-47*	*-8*	*-10*

These data suggest that even elite distance runners were unable to habituate sufficiently to treadmill running. They were subsequently excuded from the main experiment if blood lactate concentrations ($[La^-]_B$) increased by more than 2 $mmol.L^{-1}$ from rest to running at 6-8 $km.hr^{-1}$ (2.8% gradient). Thus, the aim of the present investigation was to quantify the physiological implications of habituation on treadmill performance.

METHOD

Two subjects (1 male/1 female) volunteered for this study. Both subjects were healthy and did not participate in any form of structured training. A summary of anthopometric and $\dot{V}O_{2max}$ data prior to experimentation are illustrated in Table K_2.

Table K_2 Anthropometric and Maximal Oxygen Uptake ($\dot{V}O_{2\,max}$) Data

Variable	Subject GR	Subject JR
Sex	Female	Male
Age (Yr)	23	23
Stature (m)	1.63	1.83
Body Mass (kg)	54.8	83.0
BMI (kg/m^2)	20.6	24.8
Body Fat (%)	20	18
$\dot{V}O_{2max}$ (L.min-1)	2.07	4.02
$\dot{V}O_{2max}$ ($ml.kg.^{-1}min.^{-1}$)	37.8	48.4

Each subject reported to the laboratory in a fasted state at 09:00 hr on Monday, Wednesday and Friday and were instructed to maintain normal dietary and activity patterns throughout the duration of a 2 wk study. A standardised $\dot{V}O_{2max}$ test (unpublished data, British Olympic Medical Centre) was conducted one day prior to and one day following completion of the experimental tests.

The protocol utilised during the experimental sessions is outlined in Figure K_1. This procedure was designed to minimise any potential training effects which would subsequently confound experimental conclusions. Subjects were instructed to perform two 90 s sprints at a running velocity that equated to 110% of the velocity obtained at $\dot{V}O_{2max}$ or Max_{vel} (Male subject - 18.7 $km.hr^{-1}$/ Female subject - 16.3 $km.hr^{-1}$). Subjects remained seated immediately after the first sprint for a 5 min period with their feet raised parallel to the ground to aid recovery and limit orthostatic hypotension. Exercise resumed at an intensity equivalent to 40% Max_{vel} for 17.5 minutes (Male subject - 6.8$km.hr^{-1}$/ Female subject - 5.9 $km.hr^{-1}$). Following a one minute standing recovery subjects performed a second sprint and a further 6.5 minute walk recovery. Heart rate, $\dot{V}O_2$ and $[La^-]_B$ were collected continously throughout the experimental period. Mean stride length during the sprints was calculated by counting the number of foot strikes and dividing this by the total distance covered during each sprint. Each subject completed the experimental procedure 6 times.

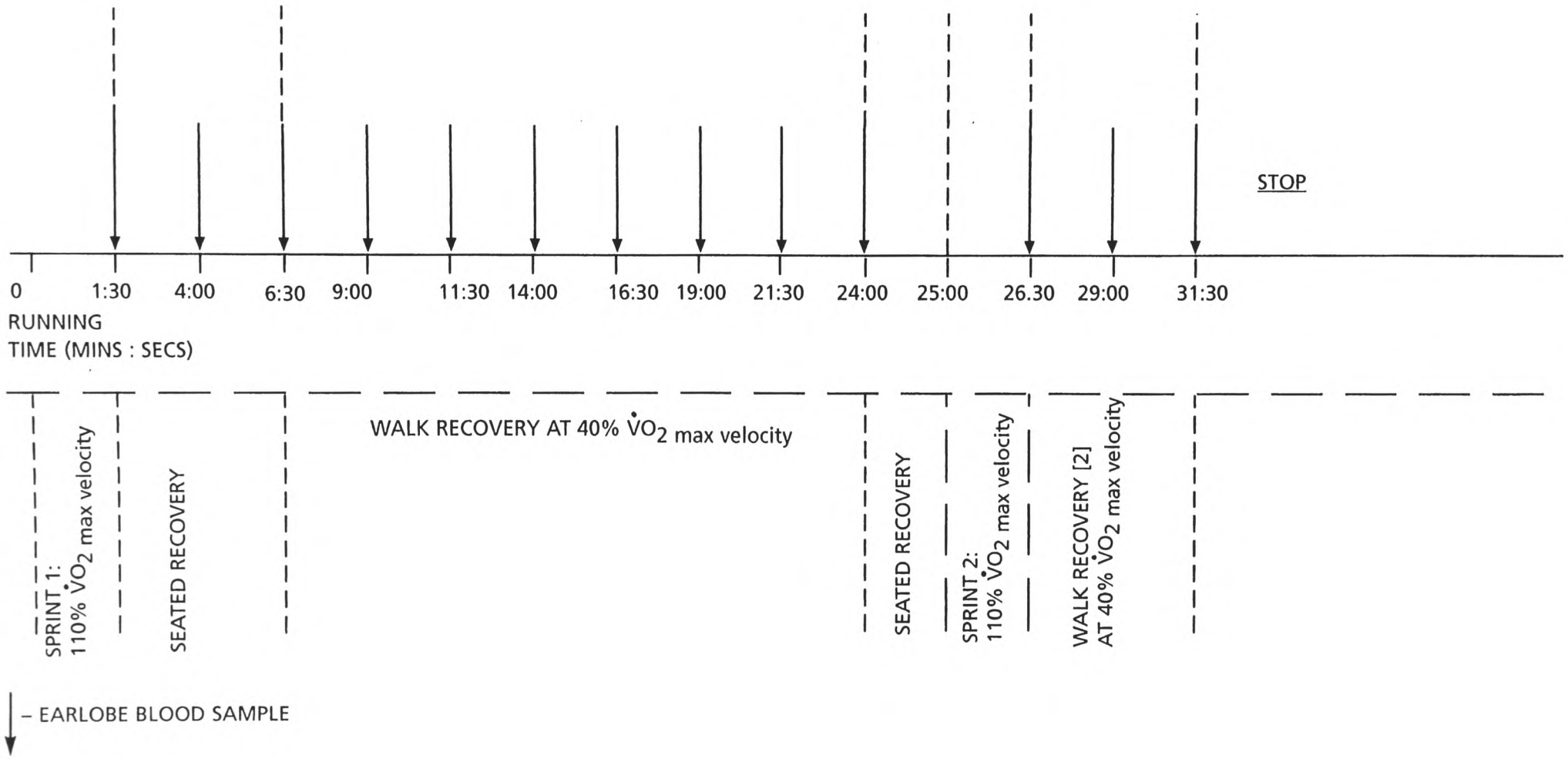


Figure K_1 Protocol for the Determination of Habituation on Treadmill Performance

RESULTS

Table K_3 summarises changes in $[La^-]_B$ and HR during the experiment. A one factor repeated measures ANOVA identified that mean $[La^-]_B$ was significantly higher during tests 1 and 2 ($P < 0.01$). Values tended to "settle" by the third test. Mean HR was significantly raised during the first test ($P < 0.01$) and plateaued by the second test. There were no marked changes in minute ventilation ($\dot{V}_E$), $\dot{V}O_2$ and stride length. Mean $\dot{V}O_{2max}$ did not change during the investigation (PRE $\dot{V}O_{2max}$ - 3.05 L.min^{-1} vs POST $\dot{V}O_{2max}$ - 3.10 L.min^{-1}).

Table K_3 Effects of Habituation on Physiological Performance

$[La^-]_B$ (mmol.L^{-1})

Activity	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6
Sprint 1	5.43	4.93	4.39	4.48	4.51	4.49
Walk	2.97	2.46	2.16	2.11	2.16	2.11
Sprint 2	4.44	3.95	3.59	3.43	3.63	3.43
X	*3.90‡*	*3.45‡*	*3.19*	*3.13*	*3.02*	*3.11*

HR (b.min^{-1})

Activity	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6
Sprint 1	134	128	124	124	124	123
Walk	134	122	118	118	117	114
Sprint 2	137	137	137	137	134	131
X	***135‡***	***127***	***128***	***124***	***123***	***120***

‡ - Significantly different from subsequent tests ($P < 0.01$)

DISCUSSION

These data highlight the potential for misinterpreting research findings if the confounding effects of habituation are ignored. It also highlights the difficulty in assessing when the subject has habituated to the task. Whilst only tentative conclusions can be made with only two subjects, it would appear that 2 treadmill runs are required to habituate a subject. Further research is warranted to clarify these findings.

APPENDIX L: ECHO-CARDIAC ULTRASOUND

NORTHWICK PARK & ST MARK'S NHS TRUST
CARDIOLOGY DEPARTMENT
ECHO-CARDIAC ULTRASOUND REPORT

Name: **Hosp No:** **EchoNo:** Misc(24)
Age: **Ward: BOMC** **Date:** 10/3/96
Sex: M **Consultant: R. Senior** **TTE Tape No:**

Intra-cardiac Dimensions	Value	Normal Adult Range
Left Ventricle (Diastolic)	5.6 cm	(3.5 - 5.6cm)
Left Ventricle (Systolic)	3.2 cm	(2.5 - 4.1cm)
Left Ventricular Fractional Shortening		(20% - 40%)
I-V Septum (Diastolic)	13 mm	(0.7 - 1.1cm)
LV Posterior Wall (Diastolic)	13 mm	(0.7 - 1.1cm)
Aortic Root Diameter	3.2 cm	(2.0 - 3.7cm)
Left Atrium	3.0 cm	(1.9 - 3.8cm)
Right Ventricle (Diastolic)	normal	(1.0 < 3.0cm)

DOPPLER VELOCITIES

Aortic Outflow	**Range**	**Mitral Flow**	**Range**	**Pulmonary Outflow**	**Range**
LVOTV =	(0.7 -1.0m/s)	**E= 80 cm/s**		**PAV=**	(0.7-1m/s)
LVOTA =		**A= 76 cm/s**			
AOV =		**PHT=**	(40 - 100m/s)	**SLOPE =**	
AOVA=	(2.2 - 2.5cm^2)	**MVA=**	(4-6 cm^2)		
SLOPE =					
PHT=				**RV-RA Gradient**	
=					

Sonographer's Findings:

Physician Report:

Mild uniform LVH with good LV function. LV size at the upper limits of normal. Valves normal. Colour flow normal.

Conclusion: The above findings are consistent with one of a trained athlete.

R Senior
R Senior

APPENDIX M: INFECTIOUS ILLNESS DATA SHEET

Incidence of Infections Date begun: 09/04/96 **Ex:** Intensive training ALTITUDE

NAME: **AGE:** 21

Ht: @1.86 **Wt:** @70/71.

Address/'Phone

Over the next week please record the occurrence and duration of infections (e.g. colds, coughs, 'flu) and/or gastric upsets (e.g. diarrhoea, vomiting - please say if B/V occurred before or after a training session. (PLEASE leave columns blank unless you are reporting an incidence of infection: if no problems just write NIL across page).

Please return questionnaire to:-

note. toilet stop on runs / not as serious as Diarrhoea but definitely more of a problem here than at home.

	Cold	Cough	Sore throat	Flu	Diarrhoea/Vomiting	Other
09/04. TUES					AM Run Toilet stop.	-also was more seriously ill last week. Diarrhoea & fever, but v/rare for me.
WED					PM Run 3x stopped for toilet	
THURS					AM Run 1 toilet stop.	
FRI						
SAT						
SUN						
MON						

APPENDIX N: SCIENTIFIC PUBLICATIONS AND PRESENTATIONS

Scientific Papers Published

Bailey, D.M. and Davies, B. (1996), Effects of habituation and passive recovery time on isokinetic leg strength. *Journal of Sport Sciences* 14 (1), 62-63.

Bailey, D.M. Davies, B. and Gandy, G. (1996), The effects of chronic altitude exposure on lung function and haematological adaptation in National standard distance runners, in Marconnet, P. Gaulard, J. Margaritis, I. and Tessier, F. (eds) *European College of Sport Science, First Annual Congress, Frontiers in Sport Science, the European Perspective* 1, 172-173.

Bailey, D.M. Davies, B. and Gandy, G. (1996), Serum urea and delta heart rate as markers of exercise intensity in National standard distance runners training at moderate altitude (1500-2000 metres), in Marconnet, P. Gaulard, J. Margaritis, I. and Tessier, F. (eds) *European College of Sport Science, First Annual Congress, Frontiers in Sport Science, the European Perspective* 1, 594-595.

Bailey, D.M. Davies, B. and Gandy, G. (1996), Physiological implications of moderate altitude training in elite distance runners (Abstract). In: *Proceedings of the 37th Annual Meeting of the Climatic Physiology Group*, Institute of Naval Medicine, Colchester, UK.

Bailey, D.M. Davies, B. Romer, L. and Gandy, G. (1996), Physiological implications of moderate altitude training (1640 metres) on sea-level endurance performance in elite distance runners. *British Journal of Sports Medicine*. Abstract 30, pp 370.

Bailey, D.M. Davies, B. Romer, L. and Gandy, G. (1996), Physiological implications of moderate altitude training (1500-2000 metres) in an elite cohort of distance runners. *British Journal of Sports Medicine* 30 (4), 370.

Bailey, D.M. Davies, B. Romer, L. and Gandy, G. (1996), The effects of moderate altitude training on serum urea and delta heart rate in an elite cohort of distance runners. *British Journal of Sports Medicine* 31 (1), 89.

Bailey, D.M. and Davies, B. (1997) The effects of altitude training on sea-level endurance performance - A review. *British Journal of Sports Medicine*, 31, 183-190.

Bailey, D.M. Davies, B. Budgett, R. and Gandy, G. (1997), Recovery from infectious mononucleosis following altitude training in an elite middle distance runner. *British Journal of Sports Medicine* 31, 153-154.

Bailey, D.M. Davies, B. Romer, L. Castell, L. Newsholme, E. and Gandy, G. (1997), The effects of 4 weeks of altitude training on plasma glutamine and endurance performance in elite distance runners, in *Proceedings of the Fifth World Congress, International Society for Adaptive Medicine*, Framingham, Massachusetts, USA.

Bailey, D.M. Davies, B. Romer, L. Castell, L. Newsholme, E. and Gandy, G. (1997), Evidence for altered immune function at altitude; physiological implications for endurance performance following return to sea-level. *Journal of Sports Sciences* 16, 36-37.

Bailey, D.M. Davies, B. Romer, L. and Gandy, G. (1997), Does chronic altitude training alter blood lipid-lipoprotein metabolism following return to sea-level in elite distance runners? In Bangsbo, J. Saltin, B. Bonde, H. Hellsten, Y. Ibsen, B. Kjaer, M. and Sjøgaard, G. (eds) *Second Annual Congress of the European College of Sports Science. Sport Science in a Changing World of Sports*, 442-443.

Godfrey, R.J. Roberts, J. Bailey, D.M. Davies, B. and Fullerton, F. (1995), Comparison of lung function values in "Elite" Male Age-Group Swimmers with "Normally Active" Schoolboys of the same age, in *Proceedings of Children in Sport, the 1st Bath Sports Medicine Conference*, July. 1, 1-5.

Scientific Papers Submitted for Publication and Presentation

Bailey, D.M. Davies, B. Romer, L. Castell, L. Newsholme, E. and Gandy, G. (1997), The effects of 4 weeks of altitude training at 1500-2000 m.a.s.l. on physiological indices of submaximal, maximal and supramaximal performance in a cohort of elite distance runners. Submitted. *European Journal of Applied Physiology and Occupational Physiology*.

Bailey, D.M. Davies, B.D. and Sanderson, D. (1997), Physiological implications of endurance training during a twin pregnancy; a case study of an elite marathon runner. Submitted. *British Medical Journal*.

Bailey, D.M. Davies, B. Romer, L. Castell, L. Newsholme, E. and Gandy, G. Altitude training: A prerequisite for success in endurance sports? To be presented at the Proceedings of the 38th Annual Meeting of the Climatic Physiology Group, Institute of Naval Medicine, Colchester, UK. September 1997.

Bailey, D.M. Biochemical adaptations to chronic hypoxia. To be presented to the Royal Society of Chemistry, University of Glamorgan, UK. January, 1998.

Scientific Papers Presented at National and International Conferences

Bailey, D.M. Davies, B. Romer, L. Castell, L. Newsholme, E. and Gandy, G. (1997), Evidence for altered immune function at altitude; physiological implications for endurance performance following return to sea-level. Presented at the British Association of Sport and Exercise Sciences, York, UK. September 1997.

Bailey, D.M. Davies, B. Romer, L. and Gandy, G. (1997), Does chronic altitude training alter blood lipid and lipoprotein metabolism following return to sea-level in elite athletes? Presented at the Second Annual Congress of the European College of Sports Science. Sport Science in a Changing World of Sports, Copenhagen, August, 1997.

Bailey, D.M. Altitude training improves sea-level endurance performance. Fact or fallacy? Presented to the Cellular Nutrition Research Group, Department of Biochemistry, University of Oxford, Oxford, UK. June 1997.

Bailey, D.M. Davies, B. and Gandy, G. The effects of moderate altitude training on serum urea and delta heart rate in an elite cohort of distance runners. Presented at the British Association of Sports Medicine, County Down, N.Ireland. November 1996.

Bailey, D.M. Davies, B. and Gandy, G. Physiological implications of moderate altitude training (1500-2000 metres) in an elite cohort of distance runners. Presented at the British Association of Sports Medicine, County Down, N.Ireland. November 1996.

Bailey, D.M. Davies, B. and Gandy, G. Physiological implications of moderate altitude training in elite distance runners. Presented at the 37th Annual Meeting of the Climatic Physiology Group, Institute of Naval Medicine, Colchester, UK. September 1996.

Bailey, D.M. Physiological implications of moderate altitude training on endurance performance at se-level. Presented to Northwick Park Medical Research Group, Northwick Park Hospital, Middlesex, UK. September 1996.

Bailey, D.M. Altitude training; physiological implications for the fitness and health of the elite competitor. Presented to the Leicester Community General Practioners Education Program, Leicester, UK. June 1996.

Bailey, D.M. Davies, B. and Gandy, G. Serum urea and delta heart rate as markers of exercise intensity in National standard distance runners training at moderate altitude (1500-2000 metres). Presented at the First European Congress of Sport Science, Nice, France, May 1996

Bailey, D.M. Davies, B. and Gandy, G. The effects of chronic altitude exposure on lung function and haematological adaptation in National standard distance runners. Presented at the First European Congress of Sport Science, Nice, France. May 1996.

Bailey, D.M. and Davies, B. Effects of habituation and passive recovery time on isokinetic leg strength. Presented at the British Association of Sports and Exercise Sciences, Queens University, Belfast. September 1995.

Bailey, D.M. The dangers of altitude training. Presented at the National Endurance Conference (British Athletics Federation), London, UK. July 1995, 1996.